BREAST DENSITY, RACE, AND INTRINSIC SUBTYPES OF BREAST CANCER

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ABSTRACT
HILDA RAZZAGHI: Breast density, race, and Intrinsic Subtypes of Breast Cancer
(Under the direction of Melissa A. Troester, PhD)

Mammographic breast density, a measure of fibroglandular tissue in the breast, refers to radiographically dense areas on a mammogram, and is among the strongest risk factors for breast cancer. Women with the highest mammographic density may be at a four- to six-fold increased risk of developing breast cancer compared with women with less dense tissue. Although the strongest risk factor, breast density is poorly understood. Whether breast density and breast cancer risk differ by race or depending upon molecular characteristics of the cancers is unknown.

Cases and controls were participants in the Carolina Breast Cancer Study (CBCS) Phase I or Phase II (1993 – 2001) who also had mammograms recorded in the Carolina Mammography Registry (CMR). After combining the two datasets, 491 cases with mammograms and 528 controls with mammograms met selection criteria. Mammographic density was reported to CMR using Breast Imaging Reporting and Data System (BI-RADS) categories. In Aim 1, mammographic density was evaluated in association with breast cancer risk among all women and by race. After adjusting for confounders, a monotonically increasing risk of breast cancer was observed with increasing BI-RADS density [OR = 2.45, highest vs. lowest, (95% confidence interval: 0.99, 6.09)]. When stratifying on race, the association appeared stronger in whites. Race- and breast density-associated covariates, such as body mass index (BMI) and hormone therapy were also weak modifiers of the breast density-breast cancer association.

In Aim 2, mammographic breast density was evaluated in association with breast cancer subtypes. The expression of ER, PR, HER2, HER1, and CK5/6 was assessed by immunohistochemistry, with ER+ and/or PR+, and HER2- tumors defined as Luminal A and ER-, PR-, HER2-, HER1+ and/or CK5/6+ tumors defined as Basal-like breast cancers. The case-control odds ratio estimates were not substantially different between Basal-like and Luminal A cancers and case-only odds ratios confirmed no significant difference in risk by subtype.

In conclusion, mammographic density is associated with increased breast cancer risk, with some suggestion of effect measure modification by race and no strong evidence of etiologic heterogeneity by subtype. These data help to elucidate poorly understood patterns and may inform breast cancer prevention strategies.

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LIST OF ABBREVIATIONS

AA	African American
AJCC	American Joint Committee on Cancer
BC	breast cancer
BD	breast density
BI-RADS	Breast Imaging Reporting and Data System
BMI	body mass index
BRCA1	Breast Cancer Gene 1
CBCS	Carolina Breast Cancer Study
CK5/6	Cytokeratin 5/6
CMDS	Carolina Mammography Data System
CMR	Carolina Mammography Registry
DAB	3,3'-diaminobenzidine tetrahydrochloride
DAG	diagram/directed acyclic graph
DCIS	ductal carcinoma *in situ*
DY	mammographically dense breast with connective tissue hyperplasia (Wolfe's parenchymal patterns)
ER	estrogen receptor
F	fatty breasts
FFTP	first full-term pregnancy
HER1	human epidermal growth factor receptor-1
HER2	human epidermal growth factor receptor-2
HT	hormone therapy
IHC	immunohistochemistry
IRB	institutional review board
LRT	likelihood ratio test
M/D	mixed/dense breasts

N	fatty breast (Wolfe's parenchymal patterns)
OR	odds ratio
P	ductal patterns (Wolfe's parenchymal patterns)
PR	progesterone receptor
RR	rate ratio
SSN	social security number
TN	triple-negative breast cancer
UNC	University of North Carolina

LIST OF SYMBOLS

%	percent
#	number
≥	greater than or equal to
≤	less than or equal to

CHAPTER 1: BACKGROUND

1.1 Overview

According to the American Cancer Society, breast cancer is the most common cancer among women in the United States; it is also the second leading cause of cancer death (Cancer Fact and Figures, 2012) [1]. Breast cancer consists of a diverse group of diseases in terms of morphology, presentation, response to therapy, and molecular profile. Recent clinical studies on the molecular profiles of breast cancers have indicated that breast tumors can be classified into five prognostically relevant subtypes on the basis of gene expression patterns: Luminal A, Luminal B, HER2 over-expressing, Basal-like, and unclassified. These subtypes also show unique etiology [2]. These refinements to classification of breast cancer heterogeneity follow on long-established differences in incidence and mortality rates according to tumor estrogen (ER), progesterone (PR) receptor status, and human epidermal growth factor receptor-2 (HER2) expression [3-11]. While classification of breast cancers has been refined, uncertainty remains in the etiology of these subtypes.

One of the strongest and most consistent independent risk factors for sporadic breast cancer is breast density [12-17]. However, the association between breast density and breast cancer risk among African Americans is not well studied. There have been two studies that have evaluated mammographic density as a risk factor within racial groups [18, 19], with one concluding that race did not significantly modify the association between breast density and breast cancer risk [19] and the other suggesting that the risk of breast cancer associated with radiographic densities is higher among African American women [18]. Given that breast density-breast cancer

associations are poorly understood in African Americans and given that aggressive breast cancer subtypes are more common in this group of women, evaluating mammographic density in association with breast cancer subtype and by race would be an important contribution toward understanding and modeling the breast cancer risk.

1.2: Epidemiology of breast density

Mammographic breast density refers to the radiologic appearance of dense areas on a mammogram, which can influence the sensitivity of mammographic screening. Mammographic breast density is a measure of fibroglandular tissue in the breast and describes the appearance of radiologically dense breast tissue. On a mammogram, fat tissue appears dark, and stroma and epithelium appear light.

Breast density is one of the strongest and most consistent risk factors for breast cancer [12-17]. The concept of mammographic density as a risk factor for breast cancer evolved from the “parenchymal patterns” described by Wolfe, who associated the presence of different patterns of breast density with an increased risk of breast cancer [20, 21]. Researchers have estimated that women with the highest mammographic density may be at a four- to six-fold increased risk of developing breast cancer compared with women with less dense tissue [12, 15, 22].

1.2.1: Mechanisms

Breast density may be the least understood risk factor for breast cancer, despite the fact that increased breast density, measured from a mammogram, is one of the strongest risk factors for breast cancer [12, 15, 18, 22-32]. Even though studies have tried to define mechanisms to explain this strong association, there is not a single mechanism or explanation that is responsible for this association [33-43]. There are a

few biological hypotheses for this strong association between breast density and breast cancer risk. One of the existing hypotheses is that mammographic density is strongly associated with the amount of collagen or breast stroma [44] and another is that breast density may be a measure of the number of epithelial cells at risk, or fibroglandular growth factors [23, 45, 46]. It is also clear that genetic and environmental factors may alter breast density. Environmental factors, such as reproductive and anthropometric factors, account for only 20-30% of the variance in the proportion of breast density suggesting that there is a heritable component to breast density [43]. These effects may be through exposure to hormones and growth factors that increase proliferative activity and quantities of stromal and epithelial tissue, thereby increasing susceptibility to carcinogens and risk of breast cancer [43, 47].

In opposition to theories that support a causal role in etiology, other theories suggest that the association is through “masking bias,” wherein high density may reduce the ability to detect cancers by mammographic screening. If this concept held, then the association between breast density and breast cancer risk would be expected to disappear with longer follow-up and repeated screening, given that breast density decreases with increasing age [48]; however, studies examining this phenomenon have found increased risk of breast cancer 7-10 years after a screening exam [20, 49, 50]. Thus “masking bias" is not entirely responsible and does not fully explain the strong association between breast density and breast cancer risk.

1.2.2: Breast density measurement methods

Mammographic density can be measured quantitatively as well as qualitatively. A quantitative measure of breast density, percent mammographic density, is achieved through computer assisted methods and is the ratio of the dense area to the total area of the breast as seen on a mammogram. Percent breast density is one of the most

common ways used to measure mammographic density and is known to be a strong predictor of breast cancer risk [12, 15, 31, 51-57]. Qualitative measures used to date include the Wolfe's parenchymal pattern (includes four categories of breast patterns: the N1 breast is considered the "normal" and is composed primarily of fat, the P1 breast is composed mainly of fat with prominent ducts that appear as linear or nodular densities occupying up to 25% of the breast, the P2 breast which has prominent ducts occupying more than 25% of the breast, and the DY breast which is poorly defined as regions of densities mixed with areas of fat [18]), Tabar classification [58], and Breast Imaging Reporting and Data System (BI-RADS) [59]. In 1992 the American College of Radiology developed the BI-RADS, which is commonly used in the United States to categorize breast density [60]. BI-RADS density assessment defines four categories of breast composition including: 1) almost entirely fat 2) scattered fibroglandular densities 3) heterogeneously dense and 4) extremely dense [61].

In a meta-analysis, comparing quantitative and qualitative measurements of breast density and their association with breast cancer risk, the combined relative risk (95% CI) for the association between breast density and breast cancer risk in incidence studies was 4.08 (2.96, 5.63) for BI-RADS categories 4 vs. 1 (Extremely dense vs. almost entirely fat) and 4.64 (3.64, 5.91) for percent density of 75% vs. <5%. Although the magnitude for BI-RADS measured breast density is slightly lower than that for percent density, this qualitative measure is a strong predictor of breast cancer risk [59]. Furthermore, the reliability of BI-RADS density measurements has been studied and BI-RADS density has proven to be useful in assessing breast cancer risk [62, 63]. Ciatto *et al.* examined the intra- and interobserver reproducibility of BI-RADS density categories and concluded that average intera-observer agreement was substantial and the average interobserver agreement was moderate. Thus, categorization of breast density using BI-RADS is consistent within readers,

reasonable between readers [64], and is a valid and reliable manner in which to measure density for use in epidemiologic studies.

1.2.3: Breast density risk factors

As mentioned above, environmental factors as well as genetic factors play a role in composition of the breast tissue, and therefore, breast density. Some of the factors that are associated with breast density include age, Body Mass Index (BMI), menopausal status, and exogenous hormone use [16, 18, 19, 41, 65-73]. Age and BMI are two of the most important predictors of breast density. Breast density is known to decrease with increasing age, as explained by the Pike model of breast tissue aging [72], and the greatest declines are associated with the menopausal transition (i.e., 45-60 years) [72, 74]. According to the Pike model, breast density is highest at the time of menarche, decreases with first full-term pregnancy and is the lowest at the time of menopause [75]. Studies have shown that differences in age significantly impact the efficacy of screening mammograms due to breast density [50]. There is an inverse association between breast density and BMI; decreasing BMI is associated with increasing breast density [41, 76]. El-Bastawissi *et al.* examined the association between breast density and BMI comparing BI-RADS categories 3,4 to categories 1,2 and estimated an odds ratio of 7.1 (6.6, 7.6) for the lowest category of BMI (≤22.3) compared to highest category of BMI (≥29.8), which was the highest estimate compared with other categories of BMI (25.4-29.7 and 22.5-25.3) [41].

Family history of breast cancer also plays a role in breast density. Women with the highest breast density tend to be premenopausal, and are also more likely to have a first-degree relative with breast cancer [13]. There are also hypotheses that suggest a mechanism for parity and breast cancer involving mammographic density among premenopausal women that may be modified by body size [77]. Studies have

concluded that exogenous hormones, including Tamoxifen, are associated with reducing breast density, and the greatest reduction occurs during the first 18 months of treatment [16]. On the other hand, menopausal hormone therapy increases mammographic breast density, therefore, potentially increasing risk of breast cancer (these data have not yet been published but were presented by C. Byrne at AACR in 2010). The incidence of increased density with hormone replacement therapy is more frequent with combined estrogen/progestin hormone replacement therapy than with estrogen alone [78-86].

Diets including protein, carbohydrates, soy, and meat intake may influence the risk of breast cancer though their effect on breast tissue morphology; specifically among postmenopausal women, studies have shown a strong positive relationship between dietary intake of total meat and high-risk parenchymal patterns [70, 87]. Studies have also shown no excess risk for fat intake as well as no association between intake of vitamins and mammographic parenchymal patterns [70]. Studies of soy intake and its association with breast density have produced contradictory results with some studies suggesting a positive trend of percent densities by quartiles of soy intake [88], some suggesting an inverse relationship [89], and some suggesting no association [90-92]. Thus, there is no conclusive evidence for a relationship between diet and breast density. Given these strong correlations with many breast cancer risk factors, breast density can be thought of as a strong biomarker of risk. Given these strong correlations with many breast cancer risk factors, breast density can be thought of as a strong biomarker of risk.

The proposed study will use breast density measured within five years prior to breast cancer diagnosis as well as breast density measured within a year post diagnosis. A few studies have examined the changes that occur in breasts and mammographic findings as a result of breast cancer treatment with exogenous

hormones such as Tamoxifen and have concluded that Tamoxifen, in particular, decreases breast density. Thus, whether other breast cancer treatments affect breast density is unclear, and studies of the association between breast density and breast cancer risk have used breast density estimates obtained from mammograms many years prior to as well as after breast cancer diagnosis [93]. Furthermore, the associations between breast density, race, breast cancer risk as well as breast cancer subtypes are poorly understood.

1.3: Breast density and race

Race may be another important factor in terms of breast density but few studies have examined whether density varies by race/ethnicity and findings are inconsistent [34, 42, 94-96]. In studies of breast density comparing African American women to white women, 3 studies concluded that mammographic density is higher in African American women compared to Caucasian women [42, 95, 96]. One study concluded that African American women have lower mean density than white women [34] and one study showed no difference in density between African American and white women [94]. Table 1.3.1 presents all 5 studies that compared breast density in African American versus white women. The BI-RADS was the method of measurement for breast density in two of the studies and percent density was the measurement used for the other three studies. Results presented in the table below are adjusted for both age and BMI, which are the two most important predictors of breast density.

The association between breast density and breast cancer risk according to different racial groups, specifically African American women, is also unclear and to date only two studies have examined this association with conflicting results [18, 19]. Table 1.3.2 presents the two studies that have examined this association among African American versus white women. Wolfe *et al.* examined this association among

85 African American cases, 75 White cases, and an equal number of race-matched controls. Increased risk of breast cancer was more strongly associated with extent of densities in African American women compared to white women, although the confidence intervals were largely overlapping. Estimated odds ratio (95% CI) for the association between breast density and breast cancer risk for highest breast density (70-100% dense) was 4.8 (95% CI: 1.3, 17.7) for African American women compared to 4.2 (1.2, 14.4) for white women. For 50-69.99% breast density the estimates were 6.9 (2.0, 24.1) and 4.2 (1.4, 12.8) for African American and white women, respectively. The log-likelihood ratio test for the interaction of race and mammographic density was not statistically significant ($0.1>p>0.05$), but tended in the direction of suggesting that the risk of breast cancer associated with radiographic densities is higher among African American women [18]. However, the sample size was relatively small as evidenced by the wide confidence intervals. Furthermore, adjustments for many risk factors/confounders including BMI and parity were not accounted for in this study [18].

Ursin *et al.* conducted a case-control study with 199 African American and 280 white cases and used a quantitative measure for breast density. Although not statistically significant, this study suggesting that breast density is as strong a predictor of breast cancer risk among African American [OR: 1.66 (0.64, 4.32)] women and for white [OR: 2.56 (1.23, 5.31)] women. Furthermore, estimates per decile increase in absolute density were 1.09 (0.96, 1.25) for African Americans and 1.18 (1.02, 1.36) for white women. Thus, the results of this study were not statistically significant for African American women [19]. As mentioned and shown in tables 1.3.1 and 1.3.2, there is sparse data on the association between breast density and breast cancer risk among African American and white women and studies examining this association accounting for race are needed. Understanding whether there are differences in associations between breast density and breast cancer risk by breast cancer subtypes is necessary.

1.4: Epidemiology of Basal-like breast cancer

Triple-negative (ER-, PR-, and HER2-) breast cancer accounts for 10-17% of breast cancers and between 50-80% of these tumors express Basal markers [8-10, 97-100]; thus Basal-like breast cancers account for 8-37% of all breast cancer cases depending on the criteria used to subtype these tumors [2, 3, 5, 101-110]. To date, there is no internationally accepted definition for Basal-like cancers, given that these tumors lack ER, PR, and HER-2 expression, many studies have adopted a triple-negative definition for Basal-like cancers. Thus many studies have used the terms triple-negative and Basal-like interchangeably [111, 112] and the characteristics and risk factors identified and mentioned below may not be specific to the Basal-like subtype but more broadly associated with the triple-negative definition.

Basal-like tumors are associated with larger size, a pushing non-infiltrative border of invasion, large zones of geographic necrosis, stromal lymphocytic infiltrate, scant stromal content, lack of tubule formation, high nuclear-cytoplasmic ratio, vesicular chromatin, prominent nucleoli, high mitotic index and frequent apoptotic cells, advanced stage at diagnosis, high histologic and nuclear grade (75-100% are grade 3), poorer prognostic index, greater incidence of recurrence, distant metastasis, and poor survival. Studies have also suggested that Basal-like tumors have a tendency towards visceral metastasis, especially in the brain and lung, and are less likely to metastasize to liver, bone, and lymph nodes [104, 113-118].

Basal-like tumors have also been associated with *BRCA1* mutations and are more frequent in hereditary *BRCA1* breast tumors [4, 118-122]. These tumors are more prevalent in younger women; the average age of women with Basal-like breast cancers range from 47 to 55 [2, 100, 103, 106, 123-125]. Women with these tumors have poorer survival regardless of stage (in a study of non-Hispanic black women, those diagnosed with late-stage triple-negative breast cancer had the poorest survival

of any comparable group) [126, 127]. Mammographic features of Basal-like tumors suggest more rapid tumor progression leading directly to invasive cancer that may require adjunct imaging tools for early diagnosis [128]. Basal-like tumors present themselves as masses, display architectural distortion, and are found in association with calcifications [129, 130].

The main characteristics of Basal-like tumors include onset at a young age (<50 years) and higher prevalence among young African American women; additionally this subtype often presents as an interval cancer rather than screen-detected breast cancer, and is significantly more aggressive than tumors from other molecular subgroups. These tumors are invasive, peak risk of recurrence is between the first and third year and the majority of deaths occur in the first 5 years, following therapy. Patients with this subtype have a significantly shorter survival following the first metastatic event when compared with those with non-Basal-like subtype. A few studies have concluded that the prevalence of these tumors is higher in younger women, among African American women followed by Hispanic women with low socioeconomic status. Millikan *et al.* [11] identified some additional risk factors for the Basal-like subtype including parity, younger age at first full-term pregnancy, shorter duration of breastfeeding, lower number of children breastfed, lower number of months breastfeeding per child, and increased waist-to-hip ratio. Table 1.4.1 below presents some of these findings [6, 11, 97, 112, 131, 132].

As mentioned above, although many studies have used the terms triple-negative and Basal-like interchangeably, Basal-like tumors have distinct characteristics and to be distinguished they should be classified using five molecular markers (ER, PR, HER-2, HER-1, and CK5/6) rather than ER, PR, and HER-2 expression only [111, 112]. Given the limited knowledge and literature on Basal-like tumors, studies examining these tumors using five-marker panels are needed.

1.5: Epidemiology of Luminal A breast cancer

The Luminal A subtype of breast cancer is the most common breast cancer subtype accounting for about 45% of all breast cancers; dominating analyses considering breast cancer [133]. Luminal A subtype of breast cancer is a hormone receptor expressing breast cancer and is expressed in the more differentiated epithelial cells of the breast [5, 11, 134]. This subtype of breast cancer is often of lower grade and responds well to therapy as these tumors show the most favorable clinical features among the five subtypes [2]. They can be treated by hormone therapy since they are hormonally driven and generally have good prognosis, display favorable survival, and respond well to hormone therapy rather than chemotherapy [133, 135] . Luminal A subtype mostly affects postmenopausal Caucasian women [12, 133]. Until recently most breast cancer studies have not differentiated subtypes of breast cancer; therefore, all of the risk factors identified thus far are assumed to affect all subtypes of breast cancer. Recently, it has become clear that there is substantial etiologic heterogeneity, but most established breast cancer risk factors are also risk factors for Luminal A breast cancer.

The main risk factors for Luminal A breast cancer are concordant with the established risk factors because they represent the largest group of breast cancer tumors and have dominated most of the analysis that consider breast cancer overall. These risk factors include including increasing age, early age at menarche, late age at menopause, family history of breast cancer, and exogenous hormone use, which are associated with increased risk of Luminal A breast cancer; whereas parity and younger age at first full term pregnancy are associated with decreased risk of breast cancer. Luminal A breast cancer usually affects women at later ages (postmenopausal) [15, 136-143].

Some of these risk factors have opposite effects on Basal-like breast cancers; for example, parity and younger age at first full-term pregnancy are positively associated with Basal-like tumors, yet they are inversely associated with Luminal A cancers. Table 1.4.1 points out some of the differences in associations as mentioned [11]. The strongest differences among these subtypes are age and race related; each subtype is more prevalent in certain age and race groups.

1.6: Breast density and molecular tumor markers

Although breast density has been determined to be one of the most significant independent risk factors for breast cancer, its association with intrinsic molecular subtypes of breast cancer is unclear. To date, there has been only one study examining the association between breast density and risk of specific subtypes of breast cancer. This study has compared the association for Luminal A and triple-negative breast cancers, which is not specific to the Basal-like subtype of breast cancer [93]. Ma *et al.* conducted a case-control as well as a case-case analysis to compare Luminal A and triple-negative breast cancers and concluded that percent mammographic density was positively associated with both Luminal A and triple-negative breast cancers with no significant differences between the two tumor types [Luminal A: 2.22 (1.04-4.78), P_{trend}=0.02; triple-negative: 2.96 (1.21-7.23), P_{trend}=0.007] when comparing women with breasts with ≥60 percent density to those with <10 percent density [93]. However, given that triple negative cases include breast cancers of subtypes other than Basal-like but where IHC assays were false negatives, these results warrant evaluation in a study that uses positive staining to identify Basal-like breast cancers. The work in Aim 2 is among the first studies to examine this association using a full marker panel to identify intrinsic subtypes.

There is also limited and conflicting literature on the association between breast density and hormone receptor status of breast cancer, including estrogen and progesterone status and whether this association varies by race. Table 1.6.2 presents the studies that have examined the association between breast density and breast cancer risk by hormone receptor status and whether the studies were of case-control, case-only, or cohort design as recently reviewed in Boyd *et. al.* [144]. In addition to this recent and comprehensive review article, our review of the literature resulted in an additional publication that was not included in the recent review article [145]. Of the six case-control and cohort studies examining the association between breast density and breast cancer risk by breast cancer hormonal status and/or subtypes to date reviewed in Boyd *et. al.* [144], four observed increased risk of both ER+ and ER- tumors [93, 146-148], two observed increased risks for ER+ tumors only, and some showed stronger associations for either ER+ or ER- tumors [149, 150]. Three of the four case-control studies, also conducted case-only analysis comparing ER+/PR+ to ER-/PR- tumors, two of these studies concluded no significant difference between ER+ and ER- tumors [93, 151] and one concluded an increased risk for ER+ tumors [149]. Of the ten studies with cases only that examined whether breast density was different based on hormone receptor status all, but one [152] concluded that there were no significant differences in breast density by hormone receptor status [128, 145, 153-159]. Two of the ten case-only studies examined the association between breast density and breast cancer subtypes including the Luminal A, Luminal B, HER2, and Basal-like subtypes and concluded no significant difference in the association between breast density and breast cancer subtypes [154, 155]. Both quantitative and qualitative measures of breast density were used in these studies and majority of these studies only included estrogen receptor status with the exception of Conroy *et al.* [151], Yaghjyan *et al.*

[148], and Ma *et al.* [93] which included both estrogen and progesterone receptor status.

The contradictory findings of the studies on hormone receptor status and molecular subtypes could be due to many different factors including differences in study populations. For example, some of the studies included postmenopausal women only whereas others included both pre- and postmenopausal women. Some of the studies included white women only [151] and others included different ethnicities including African American and Asian women [93, 151]. Another contributing factor to the differences observed may be adjustment for confounders; for example, some of the studies adjusted for BMI and age [93, 146, 148, 151, 153] which are highly associated with breast density and some did not [128, 145, 147, 149]. Many of the studies adjusted for different sets of confounders which can contribute to the findings observed [93, 128, 145-149, 151, 153]. Furthermore, different measurements of breast density as well as referent category were used in these studies. Some of the studies that used percent mammographic density considered density of 60% or higher as "dense" breasts [93, 145, 147-149, 151, 153], whereas when qualitative measure of breast density was used the "dense" category could refer to a higher density than 60% (75%+) [128, 146]. These are some of the factors that could contribute to the differences observed in these studies.

1.7: Significance

Breast density is likely the least understood yet one of the strongest risk factors for breast cancer. Associations between breast density and breast cancer risk by race as well as breast cancer subtypes are not well studied and the few studies that exist appear to conflict. Some of the well known risk factors for breast cancer have opposite effects on intrinsic subtypes of breast cancer; therefore, understanding whether the

association between breast density and breast cancer risk varies by breast cancer subtypes and/or race is crucial to closing the gaps that exist in the literature and our knowledge. It is also important to understand racial disparities in breast cancer incidence and the role of breast density in these disparities.

1.8 Tables

Table 1.3.1: Breast Density and Race

Author (year)	Race (N)*	BD Metric (Method)	Age range	Results**	Comments
Habel (2007) [95]	AA (60) White (391)	Percent density (planimetry)	40-55	***Mean (95% CI) Percent Density:*** AA: 49.0 (44.6, 53.4) White: 44.1 (42.4, 45.7)	AA women had higher mean percent density than white women
Del Carmen (2007) [94]	AA (561) White (12704)	BI-RADS	-	***BI-RADS 1****:* AA: 8.4% ; White: 6.5% ***BI-RADS 4****:* AA: 8.9%; White: 12.5%	There was no difference between races (*p*=<0.0001)
Chen (2004) [42]	AA (149) White (226)	Absolute and percent density (computer-assisted)	35-64	***Mean (95% CI) Percent Density:*** AA: 31.2 (28.1, 34.3) White: 30.0 (27.6, 32.4) ***Absolute Density (95% CI):*** AA: 163.1 (143.1, 183.1) White: 132.7 (116.8, 148.6)	AA had both higher mean percent and absolute density than whites for both age groups (≤50 vs. >50) but both mean percent and absolute density difference was stronger in women ≤50.
Del Carmen (2003) [34]	AA (207) White (463)	BI-RADS	-	***Mean density:*** AA: 2.54 White: 2.66	AA had lower mean density than whites (*p*=0.0006)
El-Bastawissi (2001) [96]	AA (883) White (25339)	BI-RADS	20-79	***OR (95% CI):*** 1.3 (1.1, 1.5) for BI-RADS (3,4) vs. (1,2)	AA had greater breast density than white women (referent)

*Only African American (AA) and white women are reported in this table
**Only p-values for with adjustments for age and BMI are reported BD, breast density; BI-RADS, Breast Imaging Reporting and Data System

Table 1.3.2: The association between breast density and breast cancer risk by race

Author (year)	BC Metric (Method)	Population	Race Case/Control	Results	Comments
Ursin (2003) [19]	Percent Density	Women 35-64 in the CARE study	***White*** 280/227 ***AA*** 199/149	***Overall:**** 5.23 (1.70, 16.13) ***White:*** 2.56 (1.23, 5.31) ***AA:*** 1.66 (0.64, 4.32)	Association with risk was stronger for older than younger women (≥50 vs. <50) (p=0.05)
Wolfe (1987) [18]	Wolfe Parenchymal Pattern	160 women age 30-85	***White*** 75/75 ***AA*** 85/85	***Overall:***[$] 4.3 (1.8, 10.4) ***White:*** 4.2 (1.2, 14.4) ***AA:*** 4.8 (1.3, 17.7)	Prevalent (77%) cases: those with a mammogram within 6 months before diagnosis Incident cases (23%): women with normal mammograms >6 months before diagnosis

*Overall OR is for ≥75% vs. <1% density. Adjusted for age, BMI, age at menarche, breast cancer family history, number of full-term pregnancies, menopausal status and HRT use, and at age first full-term pregnancy

** Overall OR is for ≥60% vs. <10% density. Adjusted for age, BMI, age at menarche, breast cancer family history, number of full-term pregnancies, menopausal status and HRT use, and at age first full-term pregnancy

[$]Results are present for 70-100% vs. <25% breast density, more predictive of risk in AA. AA, African American

Table 1.5.1: Differences in associations between breast cancer risk factors and Luminal A and Basal-like breast cancers in the CBCS [11]

Risk Factors	Luminal A tumors	Basal-like tumors
Parity		
Nulliparous	Referent	Referent
1	0.7 (0.5, 1.0)	1.7 (0.9-3.0)
2	0.7 (0.6, 1.0)	1.8 (1.1, 2.1)
≥3	0.7 (0.5, 0.9)	1.9 (1.1, 3.3)
Age at first full-term pregnancy		
Nulliparous	Referent	Referent
<26	0.7 (0.5, 0.9)	1.9 (1.2, 3.2)
≥26	0.9 (0.6, 1.2)	1.5 (0.8, 2.8)
Lactation suppressant use		
Never	Referent	Referent
Ever	0.9 (0.8, 1.1)	1.5 (1.1, 2.0)
Lifetime duration of lactation		
Never	Referent	Referent
>0-3 months	0.7 (0.6, 0.9)	0.9 (0.6, 1.4)
≥4 months	0.9 (0.7-1.1)	0.7 (0.4-0.9)
Number of children breastfed		
Never	Referent	Referent
1	0.7 (0.6-0.9)	0.8 (0.6-1.2)
≥2	1.0 (0.8-1.2)	0.6 (0.4-0.9)
Average # months breastfeeding per child		
Never	Referent	Referent
0-3.9 months	0.8 (0.7-1.0)	0.8 (0.6-1.2)
≥4 months	0.9 (0.7-1.2)	0.6 (0.4-0.9)

Table 1.6.1: Existing literature on the association between breast density and breast cancer risk by breast cancer subtypes

Author (year)	BD Metric (Methods)	Population	Results	Comments
Ma (2008) [93]	Percent density	African American and white women, 35-64, who participated in the Los Angeles County component of the Women's Contraceptive and Reproductive Experiences Study (352 cases, 376 controls)	***OR (95% CI):*** [Ω] ***Luminal A (N=184)*** 2.22 (1.04, 4.78) ***TN (N=106):*** 2.96 (1.21, 7.23) ***Luminal A vs. TN:*** 1.38 (0.47, 4.01)	Odds ratios are based comparing ≥60% density to <10% density. Cases with diagnostic or pre-diagnostic mammograms within 5 years before and controls with screening mammograms within 5 years before or 1 year after their first date of contact

[Ω] Adjusted for age at mammography, first-degree family history of breast cancer, body mass index, age at menarche, number of full-term pregnancies, age at first full-term pregnancy, a variable combining menopausal status and hormone therapy use, race, and laterality of mammogram

Table 1.6.2: Existing literature on the association between breast density and breast cancer risk by hormone receptor status and study design

Author (year)	BD Metric (Methods)	Population	Case/Control	Results	Comments
Case-Control Studies					
Yaghjyan (2011) [148]	Percent density	Postmenopausal women diagnosed with breast cancer between June 1, 1989, and June 30, 2004 from the Nurses' Health Study	1042/1794	***OR (95% CI):*** [¤] ***ER+ (N=634):*** 2.94 (2.02, 4.27) ***ER- (N=157):*** 4.78 (2.42, 9.42) ***PR+ (N=551):*** 3.21 (2.17, 4.77) ***PR- (N=249):*** 3.68 (2.12-6.37) ***HER2+ (N=140):*** 2.32 (1.03, 5.22) ***HER2- (N=423):*** 2.84 (1.83, 4.4)	Results are for ≥50% vs <10% density; stronger statistically significant association between breast density and breast cancer risk observed for ER- tumors (*P*=0.04)
Conroy (2010) [151]	Mean Percent Density	Caucasian, Japanese, and women of Native Hawaiian ancestry	607/667	***OR (95% CI): ** ***ER+PR+ (N=341):*** 4.12 (2.46, 6.89) ***ER-PR- (N=50):*** 1.39 (0.41, 4.73) ***ER+PR+ vs. ER-PR-:*** 2.97 (0.84, 10.53)	Women with higher breast density have an increased risk for ER+PR+ but not ER-PR- tumors
Ding (2010) [149]	Percent Density (Cumulus)	Women 50-75 with invasive breast cancer who attended screening at the National Health Service Breast Cancer	370/1904	***OR (95% CI):*** [Ψ] ***ER+ (N=303):*** 2.94 (1.94, 4.43)	Stronger association for ER+ comparing breast with greater than 50% dense region to those with <10% density;

		Screening Program in Norwich and Norfolk, UK		***ER- (N=36):*** 1.06 (0.47, 2.38) ***ER+ vs. ER-:*** 1.45 (1.00-2.08)	increased association with ER+
Ma (2009) [93]	Percent density	African American and white women, 35-64, who participated in the Los Angeles County component of the Women's Contraceptive and Reproductive Experiences Study	352/376	***OR (95% CI):*** [Ω] ***ER+ or PR+ (N=225):*** 2.05 (1.02, 4.10) ***ER-PR- (N=127):*** 3.01 (1.29, 7.02) ***ER-PR- vs. ER+PR+:*** 1.52 (0.59-3.91)	No significant difference (*P*=0.30); cases with diagnostic or pre-diagnostic mammograms within 5 years before and controls with screening mammograms within 5 years before or 1 year after their first date of contact
Case Only Studies					
Arora (2010) [154]	BI-RADS	Patients with stage 1-3 invasive breast cancer who had a mammogram at the time of diagnosis	1323	***Luminal A:*** BI-RADS 1: 69%, 2:76%, 3: 72%, 4: 78% ***Luminal B:*** BI-RADS 1: 13%, 2: 6%, 3: 7%, 4: 10% ***HER2:*** BI-RADS 1: 4%, 2: 4%, 3: 6%, 4: 5% ***Basal-like:*** BI-RADS 1: 14%, 2: 14%, 3: 15%, 4: 8%	No difference by subtype (*P*=0.26)
Gierach (2010) [155]	Percent Density	Women with invasive breast cancer who had a pre-treatment mammogram of the unaffected breast	227	***Mean Percent Density:*** Luminal A: 27.5% Luminal B: 27.0%	No significant difference in mean percent density between Luminal A, Luminal B, HER2+, Basal-like, or

				HER2: 27.2% Basal-like: 24.6% Unclassified: 15.7%	unclassified tumors
Cil (2009) [156]	Wolfe Score	Women who underwent breast conserving surgery for invasive breast cancer for whom a pretreatment mammogram was available	335	***Low Density (N=99):*** ER-: 10 (13.3%) ER+: 65 (86.7%) ***Intermediate Density (N=107):*** ER+: 13 (16.1%) ER-: 68 (83.9%) ***High Density (N=129):*** ER-: 13 (16.5%) ER+: 66 (83.5%)	No significant difference (*P*=0.84)
Chen (2009) [145]	MRI (percent density)	80 women with unilateral invasive ductal carcinoma with complete information of ER/PR status	80	***Measured breast density:***[£] ***ER+ (N=45):*** 9.9%±7.2% ***ER- (N=35):*** 12.6%±8.9%	No significant difference between the ER+ and ER- patient groups in measured breast density
Ghosh (2008) [153]	Percent Density (Cumulus)	Postmenopausal women with invasive breast cancer who had a screening mammogram available 4 or more years before diagnosis	286	***Mean % density:***[¥] ***ER+ (N=225):*** 31.32 (28.50, 34.14) ***PR+ (N=220):*** 31.36 (28.28, 34.45)	Density was not significantly associated with estrogen (*P*=0.11) or progesterone (*P*=0.37) receptor
Yang (2008) [128]	BI-RADS	Premenopausal women 45 or younger who had been diagnosed with BC from Jan 1999 to Nov 2005 and had undergone mammography at	198	***BI-RADS 3+4:*** ***ER+ (N=93):*** 83% of patients	Breast density of more than 50% was observed in triple negative, HER2+, and ER- tumors and there were no significant differences in

		initial diagnosis.		***HER2+ (N=67):*** 90% of patients ***Triple negative (N=38):*** 84% of patients	breast density among the three groups (*P*=0.52)
Fasching (2006) [159]	BI-RADS	Women with diagnosis of invasive breast cancer who had the initial mammography conducted within the same facility	434	***OR (95% CI) for ER+ vs. ER- (referent) tumors for BI-RADS 3+4:*** 1.20 (0.61-2.34)	No significant association
Aiello (2005) [158]	BI-RADS	Women with at least one mammogram prior to their first primary invasive breast cancer within 24 months after their index mammogram	546	***OR (95% CI) for ER+ (reference) vs. ER-:***® 1.1 (0.6-2.0)	No significant association
Roubidoux (2004) [157]	BI-RADS	Women with negative results at screening mammography and clinical breast examination performed within 17 months before they were diagnosed with breast cancer	121	***OR for the association of breast density and estrogen receptor negativity:*** 1.004Φ	No association
Hinton (1985) [152]	Wolfe Score	Patients with primary, operable, invasive breast cancer who had both preoperative mammography and samples of the primary tumor	337	***Mammographic Pattern:*** ***DY:*** ER+: 129 patients ER-: 69 patients ***P or N:*** ER+: 69 patients ER-: 70 patients	DY pattern associated with greater frequency of ER+ tumors

Cohort Studies					
Ziv (2004) [146]	BI-RADS	Women with invasive breast cancers diagnosed between January 1, 1995 and July 1, 2002 who had mammograms in one of the San Francisco Mammography Registry facilities	44,811	***OR (95% CI):*** [€] ***ER+ (N=504):*** 2.11 (1.52, 2.92) ***ER- (N=118):*** 2.25 (1.18, 4.26)	Results are for BI-RADS category 4 vs. 2; No significant association between ER+ and ER- cancers and breast density by Wald test (*P*=0.73)
Olsen (2009) [147]	Similar to BI-RADS, F breasts (BI-RADS 1 and 2) and M/D breasts (BI-RADS 3 and 4)	1009 women who participated in mammography screening in Copenhagen, Denmark from 1991-2001 and were diagnosed with invasive (N=930) or DCIS (N=79) breast cancers	48,052	***RR (95% CI):*** [Φ] ***ER+(N=609):*** 2.53 (2.13, 3.02) ***ER- (N=158):*** 2.25 (1.18, 4.26)	Increased risk for ER+ in women with M/D breasts compared to F breasts

* Overall ORs are for ≥50% density vs. <10% density and are adjusted for mean age at the time mammogram, ethnicity, BMI, parity, age at first live birth, age at menarche, menopausal status, hormone replacement therapy, and family history of breast cancer

[Ψ] ORs are adjusted for age through unconditional logistic regression [£]Results are adjusted for age

[Ω] Adjusted for age at mammography, first-degree family history of breast cancer, body mass index, age at menarche, number of full-term pregnancies, age at first full-term pregnancy, a variable combining menopausal status and hormone therapy use, race, and laterality of mammogram

[Я]Adjusted for age, BMI, age at menarche, parity and age at first birth, age at menopause, alcohol consumption, and smoking status

[¥] Results are adjusted for age, BMI, hormone replacement therapy, family history, combined age at first birth and number of births

[€] ORs are adjusted for age, BMI, postmenopausal hormone use, family history of breast cancer, menopausal status, parity, and race/ethnicity

[Φ]Adjusted for age

[®]Adjusted for BMI, age at diagnosis, menopausal statu/age at menopause, age at first birth, and AJCC stage

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CHAPTER 2: SPECIFIC RESEARCH AIMS

Mammographic density is a strong, independent risk factor for breast cancer and women with higher breast density on mammography are at increased risk of developing breast cancer [1-13]. Breast density is affected by many factors including age, body mass index, race, reproductive history, exogenous estrogens, and possibly dietary factors [14-30]. Even though it is a strong risk factor for breast cancer, uncertainty remains in the epidemiology of mammographic density. Specifically, a small number of previous studies have suggested that the association between breast density and breast cancer risk may be modified by race or other race-associated variables. Whether breast density alters risk of specific breast cancer subtypes is also unknown. It is important to understand the link between breast density and breast cancer risk, within African American and white women, separately.

According to the American Cancer Society, breast cancer is the most common cancer and the second leading cause of cancer death among women in the United States (Cancer Fact and Figures, 2011) [31]. Recently, breast cancer has begun to be considered as a group of distinct diseases, rather than as a single disease. Recently identified intrinsic subtypes of breast cancer include: Luminal A [estrogen receptor (ER) positive, progesterone receptor (PR) positive, and Human Epidermal Growth Factor Receptor 2 (HER2) negative], Luminal B (ER+, PR+, HER2+), HER2-enriched (ER-, PR-, and HER2+), and Basal-like (ER-, PR-, HER2-, CK5/6+, and/or HER1+) breast cancers. Ongoing research is further stratifying breast cancer given that each breast cancer subtype has distinct natural history.

Basal-like breast cancer subtype is fast growing, shows particularly poor overall survival [32, 33], and is more prevalent among African American breast cancer cases. Basal-like breast cancers also show unique risk factor patterns, often having associations with breast cancer risk factors in the opposite direction of what is observed for breast cancer overall. On the other hand, the Luminal A subtype is the most common subtype of breast cancer with favorable prognosis and survival [34] and with risk factor profiles previously observed for breast cancers overall. Emphasizing these two breast cancer subtypes (given their disparate behavior and etiology), and with a focus on better understanding the role of race in the breast density-breast cancer association, the proposed study aims are as follows:

AIM 1.

Evaluate the association between breast density and breast cancer risk among African American and Caucasian women in the Carolina Breast Cancer Study.

Cases and controls from the Carolina Breast Cancer Study (CBCS) were linked to data from the Carolina Mammography Registry (CMR). Since 1994 CMR has been collecting prospective information on all patients' visits for breast imaging in 65 Mammography facilities in North Carolina. CBCS is a population-based, case-control study conducted in 24 counties of North Carolina. Cases from CBCS were identified from the North Carolina Central Cancer Registry, and controls were identified through Drivers' License and Medicare beneficiary lists. We used the phase I (1993-1996) and phase II (1996-2001) of the CBCS.

To address Aim 1, a case-control analysis was conducted to examine the association between breast density and breast cancer risk among African American and Caucasian women. Race, age, hormone therapy, and body mass index (BMI) were

examined as modifiers of the odds ratio for breast density in association with breast cancer. These covariates are each associated with breast density. Other established breast cancer risk factors including as covariates were parity and age at first full-term pregnancy (FFTP), menopausal status, and first degree family history of breast cancer.

AIM 2. Evaluate the association between breast density and risk of breast cancer subtypes, specifically Basal-like and Luminal A tumors. To address Aim 2, case-control analyses for Basal-like and Luminal A breast cancers vs. all controls were conducted to estimate the association between breast density and breast cancer risk for each breast cancer subtype. We also examined risk of triple-negative breast tumors [estrogen (ER), progesterone (PR), and human epidermal growth factor receptor-2 (HER-2) negative tumors] according to breast cancer subtype to facilitate direct comparisons with the only other study on the association between breast density and risk of breast cancer subtypes. Case-case analyses were used to compare odds of breast density across subtypes, comparing Basal-like to Luminal A and triple-negative to Luminal A breast cancers.

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CHAPTER 3: RESEARCH METHODS

This study examined the association between breast density and breast cancer risk among African American and white women (specific Aim 1), and estimated the association between breast density and intrinsic subtypes of breast cancer, specifically Basal-like and Luminal A subtypes (specific Aim 2). This was accomplished by linking and analyzing existing data from a population-based mammography registry and a population-based breast cancer study. Women eligible for the proposed study were North Carolina residents who were registered in the Carolina Mammography Registry (CMR) and who also participated in the Carolina Breast Cancer Study (CBCS). To identify these women, the two data sets were merged using the last four digits of social security number, first and last name, and date of birth.

3.1 Population and Participants

3.1.1 Carolina Mammography Registry

CMR is a population-based mammography registry that was originally funded by the Department of Defense in 1994 and has been supported by the National Cancer institute since 1995 as part of Breast Cancer Surveillance Consortium. Since 1994 CMR has studied performance and outcome of screening and diagnostic mammography in communities/practices in North Carolina. As of January 2010, there are data from 39 practices and 65 participating facilities in the CMR, geographically located in eastern, western and central North Carolina. CMR collects data prospectively from both women and radiologists/technologists. Data collected from the patients and used for this study included age at the time of the mammogram and current hormone therapy use which

are taken at each time of visit. The radiologist and technologist record breast imaging data including breast density which is used in this study. CMR is also linked annually to the North Carolina Central Cancer Registry as well as breast pathology data.

Data collected for CMR is entered into data systems at the practice site (either using the Carolina Mammography Data System (CMDS), a system created and supported by CMR staff, or a commercial system). CMDS includes quality control features such as double-entry of some of the patient's information and required entry of key variables such as date of birth. After data are downloaded to CMR, missing data (such as date of birth, social security number, last name, and others) and incongruous data are flagged and a report is sent to the practices for verification. Another quality-control check includes asking the facilities to double check the records where there are inconsistencies such as the assessment shows an abnormality but no further follow up is recommended. Once quality-control and data cleaning processes are complete, the records are assigned unique identifiers, and name, address, and SSN (CMR only collects the last 4 digits) are removed. The analytic data set contains only the unique ID. Once the analytic dataset is created, another level of quality control is performed on the final data including running frequencies, cross tabs to check on missing values, and identifying outliers.

All data are handled in an encrypted format at all times, using PDP encryption software. Data are stored on a secure server away from the CMR offices behind a firewall. Passwords are required to access any of the CMR files, and these are restricted to those needing to have access only. CMR is reviewed annually by the School of Medicine IRB, and holds a Public Health Service Certificate of Confidentiality.

3.1.2 Carolina Breast Cancer Study

CBCS is a population-based, case-control study that is designed to identify genetic and environmental factors for breast cancer among African American and

Caucasian residents of North Carolina. The current study draws upon CBCS cases with invasive breast cancer and controls who were recruited in the first two phases, Phase I (1993-1996) and Phase II (1996-2001), and women with carcinoma *in situ* (CIS) and population controls who were enrolled only during the later time period (1996-2001). Randomized recruitment was used to over-sample younger (<50 years of age) and African American women [1]. The CBCS recruitment study area included 24 counties in eastern and central parts of the state [2, 3]. The study areas included suburban, small town, and rural area; county selections were based on five criteria: 1) appropriate numbers of breast cancer cases; 2) no patient referral patterns to hospitals outside of the study area; 3) population of low mobility; 4) good representation of rural residents and African American; and 5) compliance of hospitals with submission of cancer incidence data. Cases were identified from the North Carolina Central Cancer Registry using rapid case ascertainment and included women between the ages 20 and 74 residing in the 24 counties who were diagnosed with invasive breast cancer for the first time during May 1, 1993 and December 31, 2000; CBCS includes women who were between 20-74 of age at the time of their first diagnosis of a first primary breast cancer. Controls were identified using a list from the Division of Motor Vehicles (DMV) for those younger than 65, and a list from the Health Care Financing Administration (HCFA) for those 65-74. Controls were from the same 24-county geographic area as the cases and matched to the expected age distribution of the case women. Furthermore, a priori sampling fractions based on 5-year age group and race, were used to make sure that controls were frequency matched to the cases on race and age. The sampling fractions included: 100% of younger Black women, 75% of older Black women, 67% of younger White women, and 20% of older White women with younger being defined as 20-49 years of age and older defined as 50 to 74 years of age [2-5].

Prior to patient contact, a letter was sent to the physician providing cancer care requesting permission to invite the woman to participate in the study. Potential participants with physician permission were contacted first by letter and then by a telephone call. If a woman agreed to participate, an appointment was scheduled for in-person interview at the woman's home or other, agreed-upon location. Home visits and interviews for cases and controls were conducted by registered nurses and the interviews lasted about 1-1.5 hours. The trained female nurse-interviewers were matched with subjects on race for those aged 50 years or older. Interviewers administered a structured questionnaire that included detailed information about family history of cancer and reproductive history including age at menarche, age at first full-term pregnancy, number of children, breastfeeding, age at menopause, oral contraceptive use, and hormone replacement therapy. Additionally, body measurements including waist and hip circumference and weight were obtained at the time of the interview, and 30 cc blood samples were collected at the time of the interview. For cases, consent for retrieving tumor tissue, pathology reports, and medical documentation was obtained at the time of the interview. For interviewed cases, pathology reports and paraffin-embedded tissue bocks were retrieved and were reviewed by the study pathologist in a standardized way to confirm diagnosis and to describe histologic characteristics of the breast cancer [2-5].

Phases I and II of CBCS for the study of invasive breast cancer included 1,803 cases (787 African American and 1,016 white women) and 1,564 controls (718 African American and 846 white women). Furthermore, the carcinoma *in-situ* study section of the CBCS (all women with microinvasion to a depth of 2 mm, and lobular carcinoma *in* situ were eligible) included 508 cases (107 African American and 401 white women) and 458 controls (70 African American and 388 white women). The overall contact rate (contacted/eligible) was 97.6% among cases and 80.9% among controls. The

cooperation rates (enrolled/contacted) were 78.0% for cases and 70.3% for controls. The overall response rates (product of contact and cooperation rates) were 76.0% for cases and 55.0% for controls. Overall response rates for cases were lowest for African Americans age 50 and over (69.9%) and highest for whites less than age 50 (81.2%). Overall response rates for controls were lowest for African Americans less than age 50 (47.1%) and highest for whites age 50 or older (64.9%) [3, 6].

The contact rates for *in-situ* cancers were 99.3% for cases and 90.6% for controls. The cooperation rates were 83.4% for cases and 73.0% for controls. The overall response rates were 82.7% for cases and 65.2% for controls. Overall response rates among cases ranged from 76.5% in African Americans under age 50 to 86.2% in whites under age 50. Overall response rates among controls ranged from 51.0% in African Americans over age 50 to 68.9% in whites over age 50 [6].

Approval for release of formalin-fixed, paraffin-embedded tumor tissue blocks was obtained for 94% of the cases. Patients with smaller tumors/early stage were less likely to provide blocks because they were either unavailable or had insufficient tissue for subtype analysis. Included cases were less likely to be stage I (39% vs. 48%) and more likely to be stage II (51% vs. 39%), with little differences observed in stage III (8% vs. 10%) or stage IV (3% vs. 4%). There were no differences between the included and excluded cases in age, race, menopausal status, lymph node status, nuclear grade, histologic grade, or survival [2, 4, 6].

3.1.3 Data Acquisition

Permission to use the data was obtained from Dr. Robert Millikan (CBCS) and Dr. Bonnie Yankaskas (CMR). Additionally, IRB approval was obtained for merging and analyzing the data.

3.2 Merged data for the proposed study

Both invasive and CIS cases and controls from phase I, phase II, and women in carcinoma *in situ* part of the CBCS were linked with the entire CMR using probabilistic linkage [7-9] and using 4 variables/fields (last four digits of social security numbers, first and last name, and date of birth). The merge was done by the CMR programmer who has experience in merging the CMR data with other sources of data (i.e. ovarian cancer data or the National Death Index). The software used to conduct the probabilistic linkage was AUTOMATCH version 3.0 (Matchware Technologies Incorporated), and four passes were conducted to ensure validity of the match. The threshold values were calculated and set at 10 and 20, where any matches with a score of 20 or higher were considered perfect and those with a score less than 10 were considered non-matches. The links that had a score between 10 and 20 were reviewed for determining whether they were matches or not and whether there was a clerical error in entering the data. The first pass used the last four digits of social security number to link the two data sets. The second pass used last name, first name, and the last four digits of the social security number. The third pass linked the two files using last name, month and year of birth date, and the last four digits of the social security number. The last pass used first name, month and day of birth date, and the last four digits of the social security number. Two reviewers (the programmer and the author) independently examined all discordant records to determine true matches.

Figure 3.2.1 shows the areas where both Carolina Breast Cancer Study and Carolina Mammography Registry covered; the areas with pink dots are those in common to the two studies. The following counties from the CBCS were not represented in our study due to no matching cases and controls in the CMR: Alamance, Orange, Wake, Johnston, Lee, Harnett, Bertie, Wilson, Edgecombe, Pitt, Pamlico,

Beaufort, and Tyrell. Cases from the University of North Carolina Hospitals were excluded since this hospital system is not one of the CMR facilities.

Once the merge was completed, a dataset stripped of personal identifying information was created. Using the unique identification numbers from CMR and CBCS the data set was compiled and each of the patients was given a new unique identification number for the purpose of this study. The unique identification numbers for CMR and CBCS were deleted upon assigning the new identification numbers particular to this study so that the patients could not be traced back in either one of the data sources.

Table 3.2.1 presents the variables, how they were measured, and their values for this study. The quality of the data was checked by examining distributions, ranges, outliers, and missing or out of range values. If there were such values, these data were cross-checked with the original data and observations with conflicting data were deleted. We also deleted 4 (0.4%) observations with missing values for the exposure of interest, breast density. Below are the variables, their definitions, and how they were collected/measured for this study.

3.2.1 Breast density

Breast density was the main exposure and was categorized as a qualitative measure (BI-RADS density), based on analysis of film mammograms. Studies have concluded that BI-RADS breast density is as predictive of breast cancer risk as quantitative measure, percent breast density. As mentioned previously, in a meta-analysis, McCormack et al. estimated a combined relative risk of 4.64 (3.64, 5.91) for percent density of ≥75% vs. <5% and a combined relative risk of 4.08 (2.96, 5.63) for BI-RADS categorized breast density of category 4 vs. 1 [10]. Additionally, as explained in detail in the background section, fat tissue appears dark on a mammogram, and the

dense stroma and epithelium appear light. Breast density is measured as the ratio of the dense area to the total areas of the breast as seen on a mammogram. A radiologist visually assesses the mammograms, using BI-RADS criteria to categorize breast density into four categories: 1) almost entirely fat, 2) scattered fibroglandular densities, 3) heterogeneously dense, and 4) extremely dense. Breast density is measured by radiologists in each of the facilities that are a part of the CMR. As mentioned earlier, although a subjective measure, the reliability, as well as intra- and interobserver reproducibility, of this measure has been studied and categorization of breast density using BI-RADS has been feasible and predictive of breast cancer risk [11-13]. There is potential for exposure non-differential exposure misclassification due to subjectivity of the measurement tool, but the misclassification is most relevant for the two intermediate categories. The best reliability estimates have been observed for the extremes of BI-RADS (i.e., categories 1 and 4) [10].

3.2.2 Breast cancer subtypes

For Aim 1, breast cancer was the outcome of interest and for Aim 2 specific breast cancer subtypes, Basal-like and Luminal A, were outcomes. Breast cancer cases for the CBCS were originally obtained from the North Carolina Central Cancer registry. Tumor blocks and pathology reports were obtained from eligible cases and examined in order to determine breast cancer subtype. There were several different assays conducted for CBCS; the following explain how the 5 subtypes were determined.

All breast cancers underwent pathology review. Descriptive data, including type of biopsy, tumor size, laterality, number of foci, and involvement of adjacent or distant tissues, were abstracted from pathology reports. Three H&E-stained slides were produced from each of the paraffin blocks when slices were made for molecular and immunohistochemical analyses. These slides were reviewed in a standardized fashion

by the study pathologist to confirm the diagnosis of breast cancer, to assign histologic classification, and to describe features in more detail, including those characteristics recognized as prognostically significant [5]. The details are as follows.

The following markers were used to determine breast cancer subtypes: Luminal A (ER+ and/or PR+, HER2-), Luminal B (ER+ and /or PR+, HER2+), Basal-like (ER-, PR-, HER2-, HER1+ and/or cytokeratin (CK) 5/6+), HER2+/ER- (ER-, PR-, HER2+), and unclassified (negative for all five markers) [2, 4]. IHC profiles were developed by performing both tissue microarray analysis and IHC for ER, HER2, HER1, and Cytokeratin 5/6 on a single series of breast cancers [4, 14, 15]. Commercially available antibodies were used and Table 3.2.2 presents the panel of antibodies used for the Carolina Breast Cancer Study. To determine estrogen/progesterone (ER/PR) status, tumor blocks were sectioned and stained for a panel of IHC markers at the Immunohistochemistry Core Laboratory, University of North Carolina (UNC). For invasive cases, estrogen and progesterone receptor status were obtained from medical records for 80% of cases and determined using IHC assays performed at UNC for the remaining cases. For the 80% of cases with ER/PR status in the medical record, the status was determined in various clinical laboratories, in the vast majority of cases using an immunohistochemical method with cutoffs for receptor positivity. These cutoffs ranged from more than 0 to more than 20 percent for assays performed on paraffin-embedded tissues (about half) and from 10 to 15 fmol/mg for assays performed on frozen tissues (about half). For 11% of the cases with missing status for ER/PR on medical records, paraffin-embedded tissues were used and ER/PR status was determined at the UNC laboratory by using the same immunohistochemical method used by our institution for clinical purposes and a cutoff for positive assay at 5 percent. For the remaining 9 percent of the cases, ER and PR status were missing [4, 5, 16]. HER1 and Cytokeratin 5/6 were determined as follows: staining for HER1 was

categorized using a 0 to 3 scoring system, and assignment of HER1 positivity was defined as any HER1 staining, (score of at least 1) [4, 17]. Cytokeratin 5/6 was scored positive if any cytoplasmic and/or membranous staining was seen [4, 18]. HER2 membranous staining equivalent to 3+ intensity with 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen and 2+ or 3+ intensity with the SG chromogen in more than 10% of cells was scored as overexpression [15].

A few procedures were done to assure reliability of subtyping. Review of histology for all tumors was conducted by a single pathologist, Chad Livasy, for ER and PR, HER2, HER1, and CK5/6 status. The pathologist was blinded to patient demographics and all other study variables [19]. Estrogen receptor and progesterone receptor status were determined from medical records for 80% of samples and IHC was done for the remaining at the University of North Carolina-Lineberger Comprehensive Cancer Center Immunohistochemistry Core Facility in Chapel Hill [4, 16]. A comparison of a 10% random sample of 23 cases that were ER+ and 24 cases that were ER- based on medical records to those obtained through IHC done by the Core Laboratory at the University of North Carolina, resulted in a kappa statistic of 0.62, indicating agreement beyond chance [4, 20]. Even though many efforts have been made to minimize outcome misclassification, there may be misclassification introduced in determining ER/PR status since the agreement coefficient was 0.62. However, if there is outcome misclassification, we do not suspect differential misclassification.

Age at the time of selection into the CBCS

Age was collected in the CBCS at the time of interview and women age 20-74 were included in this analysis. For the purpose of this study we will use age as a continuous as well as categorizing it into 4 categories: <40, 40-49, 50-59, and ≥60. These categories are chosen based on previous studies [2].

Age at the time of the mammogram

Age was collected in the CMR at the time of the mammograms. Age at the time of the mammogram was not used in the analysis but was used to study whether there were significant differences between age at the time of selection into the CBCS and age at the time of the mammogram.

Race

Race was collected at the time of the in-person interviews for CBCS and was based on self-report. There are only two races in this study: White and African American. 1-2% of women who had reported "other" for race were excluded.

Family history of breast cancer

Family history of breast cancer was also collected at the time of the in-person interviews for CBCS. Family history is only based on whether the cases' or controls' mother or sister had been diagnosed with breast cancer and if so at what age where they diagnosed. Family history was dichotomized into yes or no.

Age at menarche

Age at menarche was determined through the interview. For the analysis for this study, age at menarche was dichotomized into two categories: <13 and ≥13 because this was the median age reported for age at menarche.

Oral contraceptive use

Oral contraceptive use was collected at the time of interview and is dichotomized into ever or never.

Age at first full-term pregnancy

Age at first full-term pregnancy was also collected through the CBCS interview and was categorized into three categories: nulliparous, <26, and ≥26 because this was the median age reported for first full-term pregnancy.

Number of live births/parity

Parity was also determined using information from the interview. We categorized parity into four categories: nulliparous, 1 child, 2 children, and ≥3 children.

Breastfeeding

This information was also determined using the CBCS interview and was based on the total lifetime number of months of breastfeeding, number of children and months each child was breastfed, and use of medication to suppress lactation. This information was used to calculate the average number of months each woman breastfed. For the purpose of this proposal breastfeeding is dichotomized into ever or never categories.

Number of children breastfed

Total number of children breastfed was determined using the interview and was categorized into 3 categories: never, 1, ≥2.

Lifetime duration of lactation

Lactation duration was collected using the interview and was categorized as: never, >0-3 months, and ≥4 months.

Menopause status

Menopausal status was determined using information from the interview. Women younger than 50 years who had undergone natural menopause, bilateral oophorectomy, or irradiation to the ovaries were classified as postmenopausal, otherwise they were classified as premenopausal. For women aged 50 or older, menopausal status was assigned based upon cessation of menstruation.

Hormone therapy (CBCS)

Hormone therapy use was only determined among postmenopausal women through the interview and is dichotomized into former, never, and current users.

Hormone therapy (CMR)

Current use of hormone therapy at the time of the mammogram was reported in the CMR. This variable was selected for the analysis because hormone therapy is highly associated with breast density and since hormone therapy was reported at the time of the mammogram where breast density was measured in the CMR, we used this variable in our analysis. Since current HT was not restricted to postmenopausal women, we conducted a sensitivity analysis using current HT in postmenopausal women. We used current HT as it was recorded in the CMR, yes or no, since the results were not substantially different between all women and post-menopausal women only.

Body Mass Index (BMI)

Body mass index was calculated based on measurements taken at the time of interview and will be calculated as weight (kg)/height (m^2) and categories of BMI are based upon National Heart, Lung, and Blood Institute cutpoints which were <25, 25-29, 30+ [21].

3.3 Data analysis

3.3.1 Addressing specific Aim 1

The main outcome variable was breast cancer for Aim 1. Univariate analysis was used to describe outcome and exposure variable distributions as well as identifying missing values and possible outliers.

To examine the association between breast density and breast cancer risk among African American and Caucasian women we conducted a case-control analysis. Odds ratios (ORs) and their 95% confidence intervals (95% CI) were calculated using unconditional logistic regression [22] as a measure of association using SAS version 9.3 (SAS Institute, Cary NC). We calculated ORs among cases and controls to examine the overall association between breast density and breast cancer risk. To account for

the sampling probabilities that were used in CBCS to oversample African American women and younger women, an offset term was included in the model.

To assess comparability of the matched dataset to the CBCS population as a whole, we conducted an analysis comparing characteristics of the matched and unmatched subject for both cases and controls in the CBCS. To evaluate whether associations were more or less similar in the subset and the CBCS as a whole, we examined whether the odds ratios obtained for each of the established risk factors for breast cancer in this study were similar to the odds ratios obtained in the whole CBCS. Results were similar, as presented in Tables 3.2.3 through 3.2.5, indicating that this subset of patients is a representative sample of the CBCS.

3.3.2 Exposure assessment

Mammograms within five years prior to and one year post breast cancer diagnosis for cases, and within five years prior and three year post to selection date for controls were used to measure breast density. Studies have shown that elevated risk of breast cancer associated with breast density persists for at least 5 years after a mammogram is taken, with studies showing persistent effects for 10 years or more for both pre- and postmenopausal women [23-28]. Since the proposed study is using a qualitative measure of breast density (BI-RADS), it is unlikely that breast density in women changes from one category to another over a short period of time. The Pike model on aging of breast tissue describes the process of aging breast tissue and changes in breast density, with greatest changes occurring with first full-term pregnancy and at the time of menopause [29]. If women had multiple mammograms prior to breast cancer diagnosis or selection date into CBCS, the mammogram closest in time to the diagnosis or selection date was chosen. Furthermore, mammograms prior to diagnosis or selection took priority, and when women did not have a mammogram prior to the diagnosis or selection date, then the mammogram

after and closest to the diagnosis or selection date was used. Studies have shown that elevated risks of breast cancer associated with breast density persist for at least 5 years after a mammogram [23-26, 28]. Breast density measured in the CMR is per woman and not per breast. Vachon *et al.* concluded that density is a general marker of breast cancer risk and is not specific to breast side or location of the eventual cancer [30]; density has also been shown to be highly correlated between breasts within a woman [31].

Many variables were available in both CMR and CBCS. We selected all variables from the CBCS to maintain consistency with previous case-control analyses using this dataset; however, for some variables highly associated with breast density, careful consideration was given as to which data source to use. In the case of age and age-related breast density changes, we compared age at the time of the mammogram (from the CMR) to age at the time of diagnosis or selection into the CBCS. The mean age was not substantially different in the CBCS and the CMR as presented in the results, therefore, we used age from the CBCS for consistency with other CBCS studies. We also examined current hormone therapy at the time of the mammogram from the CMR since current use of HT is highly associated with breast density. As mentioned earlier the sensitivity analysis using current HT in postmenopausal women only did not result in substantially different estimates, leading to using current HT as it was recorded in the CMR rather than HT from CBCS. Breast density was analyzed as a categorical variable based on the four BI-RADS categories as explained in section 3.2.5. The analyses were conducted using both BI-RADS 1 and 2 as the referent groups given the small sample size and small number of women (sometimes as low as 1) in the BI-RADS 1 group.

3.3.3 Effect measure modification

Potential effect measure modifiers were identified *a priori* and were evaluated for Aim 1 by comparing models with an interaction term and the main exposure and

outcome effects with models containing the main effects only. These variables include BMI, age, race, and hormone therapy. We examined effect measure modifiers using likelihood ratio tests [32] and a cut point of 0.05. For Aim 2 we had a smaller sample size, especially for the Basal-like subtype (N=48); therefore, we did not examine effect measure modification in the analysis for Aim 2.

3.3.4 Confounding

To determine the estimates for the outcome-exposure relationship, a single logistic regression model using backward elimination was derived to address the association of interest.

In addition to effect measure modifiers, potential confounders were selected based on the available literature and conceptual diagram/directed acyclic graph (DAG) as shown in figure 3.3.4.1 [33]. Based on the literature and our DAG, we examined age, age at menarche, age at first live birth, age at menopause/menopausal status, BMI, family history of breast cancer, parity, use of hormone therapy, and breastfeeding as potential confounders (if any of the variables examined for effect measure modifier were indeed effect measure modifiers, we did not examine them as potential confounders). Selected variables were examined using likelihood ratio tests [32, 34]. Additionally we conducted tests for trend for ordinal variables by calculating *P*-values for the beta coefficient in logistic regression models [32].

3.3.5 Addressing specific Aim 2

To evaluate whether mammographic density was associated with risk of specific subtypes of breast cancer (Luminal A and Basal-like breast cancers), we conducted two sets of analyses; case-control analysis and case-case analysis. The same steps as used for Aim 1 were used for case-control analysis. Odds ratios among cases and

controls were estimated to examine the overall association between breast density and breast cancer with respect to other risk factors for breast cancer, as well as investigating the etiology of Basal-like and Luminal A breast cancers. As an added analysis for comparison purposes, we examined the association between breast density and the risk of triple-negative breast cancers which we defined as those tumors that are ER-, PR-, and HER2-. Additionally, ORs and their 95% CIs among cases only were estimated to further compare the Basal-like and triple-negative breast cancers to the Luminal A breast cancers and uncover etiologic heterogeneity of the disease using Luminal A breast cancers as the comparison group [35]. The ORs from case-case analysis, which can be interpreted as ratios of ORs between the two subtypes of breast cancer (Luminal A and Basal-like subtypes), estimated the relative strength of association between the two breast cancer subtypes. Thus, these ORs, estimated the association between breast cancer risk factors and Basal-like subtype versus the same risk factor and Luminal A subtypes. Case-case analyses were associated with the following assumptions; 1) there is no confounding or selection bias by stage at diagnosis, which will be accounted for by adjusting case-only odds ratios for stage at diagnosis 2) the study is a population-based series of incident cases 3) marker is conditional on disease status (having a tumor) 4) exposure is related to marker status in some causal way 5) the analysis is exploratory only (the case only odds ratios measure a ratio of odds ratios and does not estimate a risk ratio) [35].

Through use of the variables and methods described here, the merged data from CBCS and CMR were used to evaluate the association between breast density and breast cancer risk in white and African American women as well as evaluating the association between breast density and Basal-like and Luminal A breast cancers.

3.4 Tables and Figures

Table 3.2.1: Variables and their description for the proposed study

Variable	Definition	Data Source
BI-RADS Breast Density (main exposure)	Categorized as Almost entirely fat, Scattered fibroglandular densities, Heterogeneously dense, and Extremely dense	CMR
Breast Cancer Subtype (main outcome)	Categorized as Luminal A, Luminal B, Basal-like, HER2+/ER-, and unclassified	CBCS
Age at diagnosis	Continuous	CBCS
Age at mammogram	Continuous	CMR
Race	Categorized as African American or White	CBCS
Family History of Breast Cancer	Categorized as Yes or No (1 or more first degree relatives with breast cancer)	CBCS
Age at first birth	Continuous	CBCS
Age at menarche	Continuous	CBCS
Age at first full-term pregnancy	Continuous	CBCS
Number of live births	Total number of live births	CBCS
Breastfeeding	Categorized as Ever or Never	CBCS
Lifetime duration of lactation	Continuous	CBCS
Age at menopause	Continuous	CBCS
Oral contraceptive use	Categorized as Ever or Never	CBCS
Hormone therapy use	Categorized as Current, Former, and Never	CBCS
Hormone therapy use at the time of the mammogram	Categorized as Yes or No	CMR
Body Mass Index (BMI)	Categorized as <25 normal or underweight, 25-29 overweight, ≥30 obese	CBCS
ER status	Categorized as positive or negative	CBCS
PR status	Categorized as positive or negative	CBCS
HER2 status	Categorized as positive or negative	CBCS

Table 3.2.2: Panel of antibodies used in CBCS for determining breast cancer subtypes

Antibody	Clone	Dilution	Company	Chromophore
ER	SP1	1:100	Dako	DAB
PR	1E2	1:100	Dako	DAB
HER2/neu	CB11	1:100	BioGenex, San Ramon, CA	DAB
Cytokeratin 5/6	D5/16B4	1:50	Zymed	SG
HER1 (EGFR)	31G7	1:7	Zymed	DAB

Table 3.2.3: Comparing Odds ratios (OR) and 95% confidence intervals (CI) for some of the established breast cancer risk factor between women in this study and the entire CBCS

Variable	CBCS OR (95% CI)	This study OR (95% CI)
Age	1.05 (1.04-1.07)	1.02 (1.00-1.04)
BMI	0.98 (0.96-0.99)	0.99 (0.97-1.01)
Race		
White	1.00	1.00
Non-white	1.76 (1.44-2.14)	1.44 (1.06-1.94)
Menopausal Status		
Premenopausal	1.00	1.00
Postmenopausal	0.77 (0.58-1.03)	1.36 (0.88-2.11)
Family history of breast cancer	1.78 (1.39-2.28)	1.32 (0.92-1.90)
Age at menarche		
<13	1.00	1.00
≥13	0.87 (0.73-1.03)	0.68 (0.52-0.90)
Parity (vs. nulliparous)		
Nulliparous	1.00	1.00
Parous, <26	0.84 (0.65-1.10)	0.94 (0.63-1.39)
Parous, 26+	0.97 (0.72-1.30)	0.86 (0.55-1.37)
Hormone therapy		
Never	1.00	1.00
Current	0.97 (0.76-1.24)	0.73 (0.51-1.05)
Former	0.95 (0.71-1.28)	0.60 (0.37-0.95)

Table 3.2.4: Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with BI-RADS measured mammographic density by race: comparison of controls groups with mammograms within 5 years prior and 1-3 years post to selection date into the CBCS

BI-RADS Categorized Density	Cases	Controls	Controls (-5, +1)[a]	Cases	Controls	Controls (-5, +2) [a]	Cases	Controls	Controls (-5, +3) [a]
All women									
Entirely Fat	13	16	0.46 (0.20-1.10)	13	23	0.45 (0.20-1.03)	13	25	0.48 (0.22-1.08)
Scattered Fibroglandular Densities	183	138	1.00 (Referent)	183	175	1.00 (Referent)	183	197	1.00 (Referent)
Heterogeneously Dense	232	154	1.10 (0.78-1.56)	232	197	1.11 (0.80-1.52)	232	253	1.00 (0.73-1.35)
Extremely Dense	63	32	1.25 (0.71-2.20)	63	47	1.20 (0.72-2.00)	63	53	1.19 (0.72-1.95)
			P_{trend} = 0.13[b]			P_{trend} = 0.13			P_{trend} = 0.24
White Women									
Entirely Fat	5	7	0.34 (0.09-1.29)	5	9	0.35 (0.10-1.31)	5	10	0.37 (0.10-1.35)
Scattered Fibroglandular Densities	98	80	1.00 (Referent)	98	97	1.00 (Referent)	98	108	1.00 (Referent)
Heterogeneously Dense	144	111	1.03 (0.66-1.60)	144	135	1.01 (0.67-1.54)	144	171	0.93 (0.63-1.39)
Extremely Dense	50	23	1.53 (0.78-3.00)	50	31	1.39 (0.74-2.61)	50	35	1.39 (0.75-2.55)
			P_{trend} = 0.12			P_{trend} = 0.18			P_{trend} = 0.23
African American Women									
Entirely Fat	8	9	0.55 (0.17-1.75)	8	14	0.45 (0.16-1.32)	8	15	0.49 (0.17-1.40)
Scattered Fibroglandular Densities	85	58	1.00 (Referent)	85	78	1.00 (Referent)	85	89	1.00 (Referent)
Heterogeneously Dense	88	43	1.25 (0.70-2.21)	88	62	1.20 (0.72-2.00)	88	82	1.02 (0.63-1.66)
Extremely Dense	13	9	0.66 (0.23-1.92)	13	16	0.72 (0.28-1.87)	13	18	0.75 (0.30-1.91)
			P_{trend} = 0.60			P_{trend} = 0.47			P_{trend} = 0.73

[a]Models are adjusted for age, race, BMI, menopausal status, family history of breast cancer, age at menarche, HT, and parity and age at first full term pregnancy combined, where BI-RADS category 2 (scattered fibroglandular densities) is the referent group.
[b]*P* for trend test is based on likelihood ratio test statistic and is two-sided.
BI-RADS, Breast Imaging Reporting and Data System

Table 3.2.5: Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with BI-RADS measured mammographic density by age, BMI, and HT: comparison of controls groups with mammograms within 5 years prior and 1-3 years post to selection date into the CBCS

BI-RADS Categorized Density	Cases	Controls	Controls (-5, +1)[a]	Cases	Controls	Controls (-5, +2)[a]	Cases	Controls	Controls (-5, +3)[a]
All women									
Entirely Fat	13	16	0.46 (0.20-1.10)	13	23	0.45 (0.20-1.03)	13	25	0.48 (0.22-1.08)
Scattered Fibroglandular Densities	183	138	1.00 (Referent)	183	175	1.00 (Referent)	183	197	1.00 (Referent)
Heterogeneously Dense	232	154	1.10 (0.78-1.56)	232	197	1.11 (0.80-1.52)	232	253	1.00 (0.73-1.35)
Extremely Dense	63	32	1.25 (0.71-2.20)	63	47	1.20 (0.72-2.00)	63	53	1.19 (0.72-1.95)
			P_{trend} = 0.13[b]			P_{trend} = 0.13			P_{trend} = 0.24
Current Hormone Therapy									
Yes									
Entirely Fat	3	5	0.52 (0.09-3.08)	3	6	0.53 (0.09-3.07)	3	8	0.46 (0.08-2.53)
Scattered Fibroglandular Densities	39	53	1.00 (Referent)	39	64	1.00 (Referent)	39	69	1.00 (Referent)
Heterogeneously Dense	70	64	1.46 (0.78-2.72)	70	76	1.37 (0.76-2.48)	70	97	1.13 (0.64-2.00)
Extremely Dense	17	5	5.54 (1.67-18.39)	17	7	5.37 (1.76-16.38)	17	7	5.61 (1.86-16.96)
			P_{trend} =0.005			P_{trend} =0.005			P_{trend} =0.01
No									
Entirely Fat	10	11	0.41 (0.15-1.13)	10	17	0.41 (0.16-1.05)	10	17	0.49 (0.19-1.26)
Scattered Fibroglandular Densities	142	82	1.00 (Referent)	142	107	1.00 (Referent)	142	124	1.00 (Referent)
Heterogeneously Dense	161	86	0.97 (0.63-1.49)	161	117	1.01 (0.68-1.49)	161	151	0.93 (0.64-1.34)
Extremely Dense	46	27	0.79 (0.41-1.53)	46	39	0.82 (0.45-1.49)	46	44	0.80 (0.45-1.43)
			P_{trend} =0.93			P_{trend} =0.79			P_{trend} =0.88

Table 3.2.5 (continued): Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with BI-RADS measured mammographic density by age, BMI, and HT: comparison of controls groups with mammograms within 5 years prior and 1-3 years post to selection date into the CBCS

BI-RADS Categorized Density	Cases	Controls	Controls (-5, +1)[a]	Cases	Controls	Controls (-5, +2)[a]	Cases	Controls	Controls (-5, +3)
Age									
<50									
Entirely Fat	4	2	0.83 (0.12-5.80)	4	3	0.91 (0.17-5.00)	4	4	0.78 (0.16-3.77)
Scattered Fibroglandular Densities	56	36	1.00 (Referent)	56	56	1.00 (Referent)	56	66	1.00 (Referent)
Heterogeneously Dense	110	60	1.09 (0.61-1.95)	110	86	1.25 (0.74-2.08)	110	114	1.10 (0.68-1.79)
Extremely Dense	46	22	1.25 (0.59-2.67)	46	31	1.48 (0.75-2.94)	46	35	1.45 (0.75-2.79)
			P_{trend} =0.54			P_{trend} =0.24			P_{trend} =0.27
50+									
Entirely Fat	9	14	0.40 (0.15-1.08)	9	20	0.36 (0.14-0.96)	9	21	0.40 (0.15-1.04)
Scattered Fibroglandular Densities	127	102	1.00 (Referent)	127	119	1.00 (Referent)	127	131	1.00 (Referent)
Heterogeneously Dense	122	94	1.11 (0.71-1.72)	122	111	1.04 (0.69-1.58)	122	139	0.92 (0.62-1.38)
Extremely Dense	17	10	1.52 (0.60-3.89)	17	16	1.09 (0.47-2.54)	17	18	1.06 (0.46-2.40)
			P_{trend} =0.10			P_{trend} =0.21			P_{trend} =0.46

Table 3.2.5 (continued): Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with BI-RADS measured mammographic density by age, BMI, and HT: comparison of controls groups with mammograms within 5 years prior and 1-3 years post to selection date into the CBCS

BI-RADS Categorized Density	Cases	Controls	Controls (-5, +1)[a]	Cases	Controls	Controls (-5, +2)[a]	Cases	Controls	Controls (-5, +3)[a]
Body Mass Index									
Women with BMI<25									
Entirely Fat	1	3	0.26 (0.02-3.11)	1	5	0.11 (0.01-1.18)	1	5	0.12 (0.01-1.36)
Scattered Fibroglandular Densities	55	30	1.00 (Referent)	55	33	1.00 (Referent)	55	37	1.00 (Referent)
Heterogeneously Dense	91	61	0.82 (0.44-1.54)	91	77	0.69 (0.38-1.42)	91	97	0.67 (0.38-1.19)
Extremely Dense	36	21	0.82 (0.36-1.86)	36	28	0.66 (0.30-1.42)	36	30	0.75 (0.36-1.57)
			P_{trend} =0.84			P_{trend} =0.61			P_{trend} =0.74
Women with BMI 25-29									
Entirely Fat	5	5	0.66 (0.14-3.16)	5	4	1.38 (0.27-7.10)	5	5	1.36 (0.28-6.55)
Scattered Fibroglandular Densities	49	42	1.00 (Referent)	49	59	1.00 (Referent)	49	64	1.00 (Referent)
Heterogeneously Dense	71	51	1.37 (0.71-2.64)	71	67	1.50 (0.82-2.72)	71	87	1.24 (0.70-2.20)
Extremely Dense	16	7	1.82 (0.58-5.72)	16	13	1.77 (0.66-4.80)	16	17	1.35 (0.52-3.49)
			P_{trend} =0.16			P_{trend} =0.20			P_{trend} =0.52
Women with BMI 30^{+}									
Entirely Fat	7	8	0.39 (0.12-1.31)	7	14	0.39 (0.13-1.18)	7	15	0.43 (0.15-1.28)
Scattered Fibroglandular Densities	79	66	1.00 (Referent)	79	83	1.00 (Referent)	79	96	1.00 (Referent)
Heterogeneously Dense	70	42	1.30 (0.74-2.29)	70	53	1.39 (0.83-2.35)	70	69	1.24 (0.76-2.04)
Extremely Dense	11	4	2.21 (0.59-8.25)	11	6	2.76 (0.83-9.18)	11	6	3.29 (1.00-10.83)
			P_{trend} =0.04			P_{trend} =0.01			P_{trend} =0.01

[a]Models are adjusted for age, race, BMI, menopausal status, family history of breast cancer, age at menarche, HT, and parity and age at first full term pregnancy combined, where BI-RADS category 2 (scattered fibroglandular densities) is the referent group.
[b]*P* for trend test is based on likelihood ratio test statistic and is two-sided.
BI-RADS, Breast Imaging Reporting and Data System

Figure 3.2.1: Carolina Breast Cancer Study and Carolina Mammography Registry areas merged

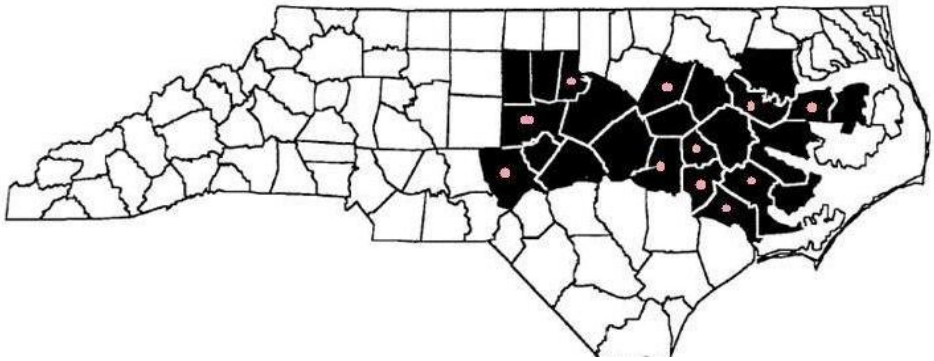
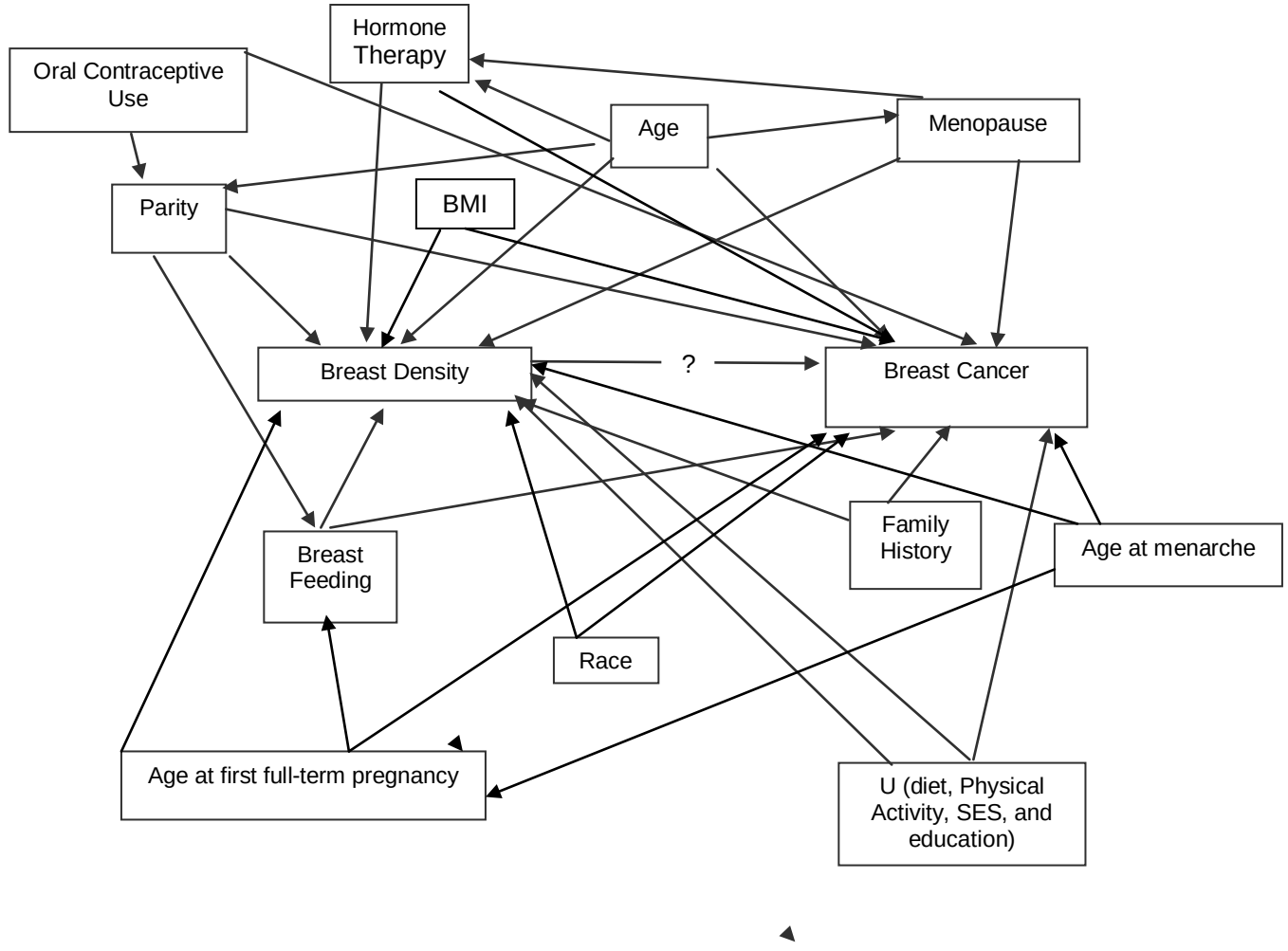


Figure 3.3.4.1: Directed Acyclic Graph for the association between breast density and breast cancer risk



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CHAPTER 4: RESULTS

4.1 Introduction

Mammographic breast density describes the radiological appearance of dense breast tissue and is a measure of the fibroglandular tissue composition in the breast. Different classification schemes have been used to visually characterize breast density, including Wolfe's parenchymal patterns [1, 2], Tabar's classification scheme [3], the American College of Radiology's Breast Imaging Reporting and Data System (BI-RADS) [4], as well as more quantitative methods that estimate the percentage of the breast that is dense. Reviews have concluded that mammographic density is associated with breast cancer risk regardless of the method used to measure breast density [5, 6]. In fact, breast density is one of the strongest and most consistent risk factors for breast cancer, and studies have estimated that women with the highest mammographic density may be at a 4-6 fold increased risk of developing breast cancer compared with women with the least dense tissue [5, 7-13].

The majority of studies examining the association between breast density and breast cancer risk have been among white women, but breast density may vary by race. Data examining the association in different racial groups, including African American (AA) women, are limited and have reported conflicting results: three studies concluded that AA women have higher mammographic density [14-16], two found no difference [17, 18], and one found lower mammographic density in AA compared with white women [19]. Only two studies have examined the association between breast density and breast cancer risk in AA women. Wolfe *et al.* reported stronger effects of breast density on risk among AA women [20], while

Ursin et al. suggested that effects were stronger among white women [11]. Variation in exposures that predict breast density and breast cancer risk may differ in prevalence by race, such as BMI and hormone use, and these factors may also play a role in the associations between race, breast density, and breast cancer risk. Ursin *et al.* did not examine hormone therapy (HT) as an effect measure modifier, but suggested potential modification of the breast density-breast cancer risk association by BMI [11]. Given strong secular trends in associations between race, BMI, and HT and because of the role of each of these factors in predicting both breast density and breast cancer risk, evaluation of these factors within a single study could help explain how these factors interact to affect breast cancer risk. Substantial disparities in breast cancer mortality exist between AA and Caucasians [21] and it is important to understand the factors that contribute to breast cancer risk in each group.

We examined the association between breast density and breast cancer by race, BMI, and HT use in the Carolina Breast Cancer Study (CBCS). The CBCS is a large, population-based study that oversampled young, AA women. By linking the CBCS with the Carolina Mammography Registry (CMR), we were able to obtain the BI-RADS density classification for a large number of women in the CBCS.

4.2 Methods

4.2.1 Study setting and population

Subjects included in this study were participants in the CBCS who also had mammograms recorded in the CMR. CBCS is a population-based, case-control study designed to identify genetic and environmental factors for breast cancer risk in AA and Caucasians. CBCS participants are residents of 24 counties in North Carolina and were recruited in two phases, Phase I (1993-1996) and Phase II (1996-2001). Cases were women with invasive breast cancer (Phase I & II) or carcinoma *in situ* (CIS, Phase II).

Controls were age and race frequency-matched to cases. Cases were identified from the North Carolina Central Cancer Registry, and controls were identified using drivers' license and Medicare beneficiary lists [22-24]. Randomized recruitment was used to over-sample younger and AA women [25]. Participants ranged in age from 20 to 74 years and provided informed consent via a protocol approved by the Institutional Review Board of the University of North Carolina School of Medicine. Response, contact, and cooperation rates for all the phases of CBCS have been published previously [26]. In person interviews were conducted for cases and controls and body size measurements including waist circumference, hip circumference, and body weight were measured by a nurse at the time of the interview [27].

The Carolina Mammography Registry (CMR) is a community-based mammography registry funded by the Department of Defense in 1994 and supported as part of Breast Cancer Surveillance Consortium by the National Cancer Institute since 1995. Since 1994, CMR has prospectively collected data in mammography practices, studying performance and outcomes of community-based screening and diagnostic breast imaging in communities and practices in North Carolina. As of January 2010, there were data from 65 participating facilities located in 39 counties, representing locations in eastern, western and central North Carolina. Data collected at the time of each imaging study include: self-reported date of birth, race/ethnicity, family history of breast cancer, menopausal status, and current HT use and imaging data recorded by the radiologists and the technologists including breast density and imaging methods. CMR is approved and reviewed annually by the Institutional Review Board of the University of North Carolina School of Medicine [28]. CMR adheres to strict confidentiality and security procedures; complies with the Health Insurance Portability and Accountability Act; and has a Federal Certificate of Confidentiality and other protections of research subjects, radiologists, and mammography facilities. The following counties included in the CBCS were not represented in our study due to no matching cases and controls in the CMR: Alamance, Orange, Wake, Johnston,

Lee, Harnett, Bertie, Wilson, Edge-Combee, Pitt, Pamlico, Beaufort, and Tyrell. CMR and CBCS were linked using probabilistic linkage with four variables; first and last name, date of birth, and last four digits of the social security number [29-31]. BI-RADS breast density, HT, and age were collected from the CMR and all other participant data were taken from the CBCS.

4.2.2 Mammographic density assessment

Mammographic breast density is recorded qualitatively in the CMR using the American College of Radiology's BI-RADS classification. BI-RADS density assessment defines four categories of breast composition including: 1) almost entirely fat, 2) scattered fibroglandular densities, 3) heterogeneously dense, and 4) extremely dense [4]. Breast density measured in the CMR is per woman and not per breast. Vachon *et al.* concluded that density is a general marker of breast cancer risk and is not specific to breast side or location of the eventual cancer [32]; density has also been shown to be highly correlated between breasts within a woman [33].

For our analysis we defined density based on the reported BI-RADS density from the screening or diagnostic mammogram that was performed within five years prior to diagnosis and up to one year after breast cancer diagnosis for cases; for controls, we selected BI-RADS density from the screening or diagnostic mammogram showing no cancer within five years prior to and up to three years after the selection date. If women had multiple mammograms prior to breast cancer diagnosis or selection date into CBCS, the mammogram closest in time to the diagnosis or selection date was chosen. Furthermore, mammograms prior to diagnosis or selection took priority when women did not have a mammogram prior to the diagnosis or selection date, then the mammogram after and closest to the diagnosis or selection date was used. Studies have shown that elevated risks of breast cancer associated with breast density persist for at least 5 years after a mammogram [8, 12, 34-36]. There are some suggestions in the literature that

agents used to treat breast cancer may alter breast density as early as 18 months after initiating therapy [37], and thus for cases, we excluded mammography exams that occurred more than one year after diagnosis.

To assess whether broader inclusion dates among controls affected comparability to cases, we conducted a sensitivity analysis with controls (n=340) restricting mammograms to five years prior to and <1 year after the control selection date. Effect estimates for the association between breast density and breast cancer risk did not differ substantially using either control group. Thus, the larger control group was used to increase precision. A total of 1,019 subjects met our inclusion criteria, representing, 491 cases and 528 controls.

4.2.3 Statistical Analysis

The variable coding schemes for covariates were chosen for consistency with previous CBCS publications [22]. Briefly, race was categorized as AA or white based on self-report. Age was age at diagnosis for cases and age at selection into the CBCS for controls and was used as a continuous variable in analyses and as a categorical variable (<50 vs. 50+) for assessment of effect measure modification by age, which is similar to the cutpoints used in previous studies of breast density and breast cancer risk. Body mass index (BMI) was calculated as body weight (kg)/height $(m)^2$ and was used as a continuous variable. To assess whether BMI was an effect measure modifier, BMI was categorized based upon National Heart, Lung, and Blood Institute (NHLBI) cutpoints (<25 normal or underweight, 25-29 overweight, and ≥30 obese) [38]. Age at first full-term pregnancy and parity/nulliparity were combined to create a categorical variable that encapsulated both parity status and age at first birth. Given the associations between age, HT use, and breast density, we also examined age and current HT use at the time of the mammogram as recorded in the CMR. There was not a significant difference between age at diagnosis/selection into the CBCS and age at the time of the mammogram as noted in Table

1; hence, age at diagnosis/selection into the CBCS was used in subsequent analyses. HT use was categorized as current vs. not current as collected by the CMR at the time of the mammogram. Since HT use was not restricted to postmenopausal women, a sensitivity analysis restricting HT users (as reported in CMR) to postmenopausal women (as reported in CBCS) was conducted. The results for HT use were not substantially different among all women vs. after restriction to only postmenopausal women, therefore, HT use was used without restrictions for menopausal status. All categorical variables were coded using indicator variables rather than ordinal variables.

We used unconditional logistic regression with breast cancer as the outcome variable to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between breast density and breast cancer risk (SAS version 9.3, SAS Institute, Cary NC). To assess the comparability of the CMR-CBCS merged data and the full CBCS dataset, we compared the characteristics of participants who matched to the CMR (the current dataset) to those in the entire CBCS by estimating ORs for established breast cancer risk factors. The ORs were similar in the CMR-CBCS merged dataset and the CBCS as a whole for all variables assessed, including first degree family history of breast cancer, current HT use, and menopausal status (data not shown).

Likelihood ratio tests were used to examine effect measure modification of the breast density-breast cancer risk association by race, BMI, HT use, and age; *p*-values of <0.05 were considered significant. Menopausal status was not examined as an effect measure modifier due to the high correlations between categories of age (<50 vs 50+). Potential confounders were selected based on prior knowledge, using directed acyclic graphs (DAGs) [39]. We adjusted for age, race, BMI, current HT, menopausal status, first degree family history of breast cancer, age at menarche, and parity and age at first full-term pregnancy combined. We also adjusted for the offset term used in the CBCS to account for the

sampling scheme used in the CBCS to oversample young AA women. The same variables were retained in models that included interaction terms for BMI, HT use and race.

4.3 Results

Characteristics of breast cancer cases and controls are presented in Table 1. The time between CBCS selection date and the date of the selected mammogram in the CMR ranged from -3.8 to 1 year with average of one month prior to diagnosis for cases and -4.4 to 3 years with an average value of 6 months post selection for the controls (Table 1). Overall and within each racial group, cases were slightly younger than controls, were more likely to have first degree family history of breast cancer, were younger at menarche, and were more likely to be never users of HT. White cases were more likely to be premenopausal, while AA cases were more likely to be postmenopausal compared to race-matched controls. White cases were more likely to be never users of oral contraceptives and AA women were more likely to be ever users of oral contraceptives (compared to their race-matched control groups). Case-control differences in age at menarche were observed in all women. Case-control differences were observed for first degree family history of breast cancer, breastfeeding and HT use in AA women.

Statistically significant differences were observed in breast density comparing AA and white women. White cases and controls had a greater percentage of “extremely dense” and “heterogeneously dense” breasts compared with AA cases and controls. The BI-RADS density category with greatest prevalence among AA was “scattered fibroglandular densities” (BI-RADS 2), while among whites “heterogeneously dense (BI-RADS 3)” was the most prevalent. In the dataset as a whole, “heterogeneously dense” was the most prevalent category; thus, when modeling the OR associated with breast cancer risk, BI-RADS density category 2 was set as the referent.

Table 2 presents the ORs and 95% CIs for unadjusted and adjusted models with both BI-RADS 1 (Model 1) and 2 (Model 2) as the reference groups. Model 1 is included to facilitate comparison with previous studies that have reported risk associations for the "extremely dense", BI-RADS 4 group, relative to the "almost entirely fatty", BI-RADS 1 group. Among all women, those with BI-RADS 4 density had an increased risk of breast cancer compared to women with BI-RADS 2 and BI-RADS 1 densities [1.19 (0.72, 1.95), and 2.45 (0.99, 6.09), respectively]. As mentioned, we conducted sensitivity analyses restricting the exposure window for mammograms with BI-RADS density results among controls, and results did not differ among the three potential groups; for example, the estimates for the association between breast density and breast cancer risk comparing BI-RADS category 4 to BI-RADS category 2 among all women was [1.25 (0.71-2.20)] for controls with the same selection criterion as cases, [1.20 (0.72-2.00)] and [1.19 (0.72-1.95)] for controls with mammograms within five years prior to and two years and three years post selection date into the CBCS, respectively. We therefore used the larger control group (with mammograms selected within 5 years prior to and 3 years post CBCS selection date) for all subsequent analyses.

Race was examined as an effect measure modifier of the breast density-breast breast cancer association using likelihood ratio tests, (p-value = 0.76). While the distribution of breast density differed by race, the effects of breast density on breast cancer risk did not differ significantly by race. However, several of the confidence intervals were wide, especially when BI-RADS density category 1 was used as the reference group. Some variation by race was evident when comparing the most dense category to the referent category of BI-RADS 2 (Model 2, Table 2). A positive association between breast density and breast cancer risk was observed among white women [OR for BI-RADS 4 vs. 2=1.39, (0.75, 2.55)], while an opposite and inverse association was observed in AA women [OR for BI-RADS 4 vs. 2=0.75, (0.30, 1.91)].

Recent literature has suggested that two important variables that differ in prevalence by race -- BMI and HT use -- may modify the association between breast density and breast cancer risk [40, 41]. Thus effect measure modification by BMI, age and HT use were considered. Menopausal status was not examined as an effect measure modifier due to high correlation with categories of age (<50 vs 50+). For example, mean BMI was 28.5 (95% CI: 15.1-60.6) among premenopausal and 28.9 (14.6-60.9) among postmenopausal women. Similar values were observed for women younger than 50 [28.7 (95% CI: 15.1-60.9)] and 50 and older [28.8 (95% CI: 14.6-57.9)]. The LRT *p*-values for interaction indicated no significant effect modification by age (0.67), or BMI (0.09), but HT use was a significant effect measure modifier (0.0002).

Table 3 shows that the associations with density were strongest among current HT users, with an almost 6-fold increase comparing current users with extremely dense breasts to current users with scattered fibroglandular densities ([OR=5.61 (1.86, 16.96)], Model 2, Table 3). Among current users of HT, there was also a significant trend (*p*-value=0.01). In contrast, among never users of HT the estimates for the association between breast density and breast cancer risk were close to null, and among former users odds ratios <1 were observed. Furthermore, there was no modification of the OR by age. Among obese women, those with extremely dense breasts had a 3-fold increased risk of breast cancer relative to those with heterogeneously dense breasts [3.29 (1.00, 10.83)], and there was a significant trend in obese women (*p*-value=0.01) (Table 3). For women with BMI less than 25, ORs for breast density-breast cancer associations were below the null, suggesting an inverse association, and for women with BMI 25-29 a very modest increased risk was observed with increasing breast density (Table 3).

4.4 Discussion

This study combined two rich data sets -- the CBCS, where young AA women were oversampled, and the CMR -- to examine modification of the breast density-breast cancer

risk relationship by age, race, BMI, and current HT use. Effect modification has not been thoroughly examined in the two previous published studies of breast density and breast cancer risk in AA and white women [11, 20]. Although the estimates were imprecise in AA women, our study found differences in the distributions of breast density between white and AA women consistent with studies conducted by Chen *et al.*, Del Carmen *et al.*, Habel *et al.*, and El-Bastawissi *et al.* [14-16, 19]. Our findings agree with Del Carmen *et al.* and Chen *et al*. [14, 19], with breast density being lower in AA women. Furthermore, increasing mammographic breast density was associated with increased breast cancer risk in CBCS, with effect estimates similar to those previously reported using BI-RADS measurements of breast density [5]. Women in the CBCS with extremely dense breasts had a nearly 3-fold increased risk of breast cancer compared to women with breasts composed of almost entirely fatty tissue. Due to the small sample size of BI-RADS density category 1, we also assessed risk using BI-RADS density category 2 as the referent group which resulted in more precise effect estimates, but with lower magnitude than reported previously by Ziv *et al* [42]. Ziv et al. was a very large study including more than 44,000 women, and estimated an OR of 2.09 (1.59, 2.75) comparing BI-RADS 4 to BI-RADS 2, which is larger than our overall OR. However, the CBCS included a large percentage of AA women (~40% of participants were AA in the present analysis), which may have attenuated the effect estimate given that the distribution of breast density varied by race.

Examining race as an effect measure modifier, our results agreed with Ursin *et al.* in that race did not significantly modify the association between breast density and breast cancer risk. However, both studies found effect estimates that were substantially weaker for AA women compared with white women. Specifically, Ursin *et al*. found a stronger association for white women compared with AA women [2.56 (1.23-5.31), and 1.66 (0.64-4.32), respectively], when comparing those with quantitative density of >60% to the lowest density (<10%) group [11]. The agreement between these two studies suggests that there

may be some weak effect modification by race, but the ability to precisely quantify this has been limited by sample size. Larger studies and meta-analyses will be needed to definitively answer this question, especially in light of conflicting reports from the only other study to date to examine the association: Wolfe *et al.* [20] reported that AA had slightly higher risk than white women in association with breast density in a study including 160 cases and 160 controls (among whom 85 cases and 85 controls were AA) and using Wolfe's parenchymal patterns as the breast density measurement tool.

Effect attenuation in AA women may be due to modification by BMI [11]. Previous studies have concluded that BMI is an important predictor of breast density and have suggested an inverse association between BMI and breast density [43, 44]. Ursin *et al.* reported that the association between breast density and breast cancer risk appeared to be modified by BMI, with a U shaped curve: the associations were highest among very thin and very obese women. The effect estimates were not presented in that paper, preventing direct comparison with our results. But more recently, Conroy *et al.* reported stronger effects of breast density on breast cancer risk in overweight and obese women compared to women with normal BMI [40]. Our findings are similar to those of Conroy *et al.* [40]. Taken together, these studies suggest that evaluation of the role of BMI in modifying risk associated with breast density merits further investigation in larger studies, and is an important consideration in studies of breast density by racial/ethnic group. However, we were unable to further stratify our race-specific models by BMI due to small sample size. While the precise mechanism underlying these associations remains to be determined, the statistical interaction between BMI and breast density with respect to risk may reflect the underlying breast tissue biology. Elevated BMI has been associated with increased inflammatory cells in breast tissue [45], and it is possible that the changing cytokine and inflammatory milieu present in obesity interacts with the fibroglandular tissue to modify the breast microenvironment and increase breast cancer risk.

We also examined HT use as a possible effect measure modifier due to its association with race, breast density, and breast cancer risk. Previous studies have concluded that HT increases mammographic breast density, therefore may increase risk of breast cancer [46-50]. This association may contribute to our race-specific effects, given that 35% of white women in our study were current HT users compared to 14% of AA women. Our findings were similar to those of Aiello *et al.* and Kerlikowske *et al.,* suggesting greater risk of breast cancer associated with elevated breast density among current users of HT [51, 52]. In examining age as an effect measure modifier, we found a slight, but not statistically significant, increased risk between BI-RADS density and breast cancer risk among younger women, where as Ursin et al. observed stronger risk associations among older women [11]. Differences between studies could relate to different exposure assessment methods. A limitation of our study is that we used a qualitative measure of mammographic density, and while BI-RADS density measures have been shown to predict breast cancer risk [5], results from studies using qualitative vs. quantitative density assessment methods may not be directly comparable. It is also challenging to merge two datasets (i.e., the CBCS and CMR) with different dates of collection. While we were unable to use BMI from the CMR, we carefully evaluated age differences between datasets and selected HT use at the time of the mammogram (choosing CMR data over CBCS data), thereby reducing exposure misclassification in studying effect modification by HT.

Given the stronger associations observed among current HT users and obese women, fewer AA women in our study may be susceptible to the strongest effects of breast density. That is, few AA women were both obese and had extremely dense breasts, and likewise, current HT use was much more common in white women (22% vs. 6% in AA women). The relatively lower number of women in the categories with strongest effects (e.g. extremely dense breasts) resulted in reduced precision for the breast density effect

estimates for AA women. Given the small sample size, we were unable to examine effect measure modification by age, BMI, and HT use within the strata of race.

However, by simultaneously considering effect modification by both race and race-associated variables, our study suggests important relationships between breast cancer risk factors and breast density. Future studies with larger numbers of AA women should fully examine the association between breast density and breast cancer risk, considering race, BMI, and HT to disentangle these factors.

4.5 Tables

Table 4.1: Descriptive characteristics of breast cancer cases and controls by race

Variable	All women		Whites		African Americans	
	Cases	Controls	Cases	Controls	Cases	Controls
No of subjects	491	528	297	324	194	204
Mean age (CBCS), y[a]	53.2 (28-74)	54.0 (31-74)	53.9 (28-74)	54.5 (35-74)	52.0 (30-74)	53.3 (31-74)
Mean age (CMR), y[b]	53.2 (28-77)	54.5 (34-76)	53.9 (28-77)	54.8 (35-76)	52.0 (30-74)	53.9 (34-76)
Mean BMI[b]	28.6 (15.1, 60.6)	28.8 (14.6, 60.9)	26.5 (17.2, 49.5)	26.8 (16.2, 52.9)	32.0 (15.1, 60.6)	32.1 (14.6, 60.9)
Mean number of days[c]	-21 (-1401, 365)	149 (-1617, 1095)	-29 (-1401, 365)	133 (-1526, 1095)	-9 (-1210, 365)	175 (-1617, 1078)
Breast density						
Almost entirely fat	13 (2.7%)	25 (4.7%)	5 (1.7%)	10 (3.1%)	8 (4.1%)	15 (7.4%)
Scattered fibroglandular Densities	183 (37.3%)	197 (37.3%)	98 (33.0%)	108 (33.3%)	85 (43.8%)	89 (43.6%)
Heterogeneously dense	232 (47.3%)	253 (47.9%)	144 (48.5%)	171 (52.8%)	88 (45.4%)	82 (40.2%)
Extremely dense	63 (12.8%)	53 (10.0%)	50 (16.8%)	35 (10.8%)	13 (6.7%)	18 (8.8%)
Menopausal status						
Premenopausal	200 (40.7%)	213 (40.3%)	120 (40.4%)	127 (39.2%)	80 (41.2%)	86 (42.2%)
Postmenopausal	291 (59.3%)	315 (59.7%)	177 (59.6%)	197 (60.8%)	114 (58.8%)	118 (57.8%)
Family history[d]						
No	386 (81.1%)	440 (85.6%)	232 (80.1%)	263 (83.0%)	154 (82.4%)	177 (89.9%)
Yes	90 (18.9%)	74 (14.4%)	57 (19.7%)	54 (17.0%)	33 (17.6%)	20 (10.2%)
Missing[f]	15	14	8	7	7	7
Age at menarche						
<13	257 (52.3%)	230 (43.6%)	148 (49.8%)	135 (41.7%)	109 (56.2%)	95 (46.6%)
≥13	234 (47.7%)	298 (56.4%)	149 (50.2%)	189 (58.3%)	85 (43.8%)	109 (53.4%)
Parity & age at FFTP[e]						
Nulliparous	74 (15.1%)	67 (12.7%)	39 (13.1%)	45 (13.9%)	35 (18.0%)	22 (10.8%)
Parous, <26	312 (63.5%)	347 (65.7%)	178 (59.9%)	200 (61.7%)	134 (69.1%)	147 (72.1%)
Parous 26^{+}	105 (21.4%)	114 (21.6%)	80 (26.9%)	79 (24.4%)	25 (12.9%)	35 (17.2%)
Breastfeeding						
Never	299 (60.9%)	324 (61.4%)	163 (54.9%)	201 (62.0%)	136 (70.1%)	123 (60.3%)
Ever	192 (39.1%)	204 (38.6%)	134 (45.1%)	123 (38.0%)	58 (29.9%)	81 (39.7%)

Lifetime duration lactation						
Never	299 (60.9%)	324 (61.4%)	163 (54.9%)	201 (62.0%)	136 (70.1%)	123 (60.2%)
>0-3 months	72 (14.7%)	69 (13.1%)	58 (19.5%)	45 (13.9%)	14 (7.2%)	24 (11.8%)
4^{+} months	120 (24.4%)	135 (25.6%)	76 (25.6%)	78 (24.1%)	44 (22.7%)	57 (27.9%)
Current HT use at the time of the mammogram						
No	359 (73.6%)	336 (65.0%)	193 (65.4%)	182 (57.2%)	166 (86.1%)	154 (77.4%)
Yes	129 (26.4%)	181 (35.1%)	102 (34.6%)	136 (42.8%)	27 (14.0%)	45 (22.6%)
Missing[f]	3	11	2	6	1	5
Oral contraceptive use						
Never	170 (34.6%)	170 (32.4%)	95 (32.0%)	89 (27.6%)	75 (38.7%)	81 (39.9%)
Ever	321 (65.4%)	355 (67.6%)	202 (68.0%)	233 (72.4%)	119 (61.3%)	122 (60.1%)
Missing[f]	0	3	0	2	0	1
WHR						
<0.77	132 (27.3%)	169 (32.3%)	110 (37.2%)	133 (41.3%)	22 (11.7%)	36 (17.8%)
0.77-0.83	171 (35.3%)	173 (33.0%)	110 (37.2%)	105 (32.6%)	61 (32.5%)	68 (33.7%)
≥0.84	181 (37.4%)	182 (34.7%)	76 (25.7%)	84 (26.1%)	105 (55.9%)	98 (48.5%)
Missing[f]	7	4	1	2	6	2

[a]Mean (range) age at diagnosis for cases and selection for controls in the CBCS
[b]Mean (range) age at the time of the mammogram in the CMR
[c]Mean (range) number of days between diagnosis date for cases and selection date for controls in the CBCS and the date of the mammogram chosen to assess breast density
[d]First-degree family history of breast cancer
[e]FFTP; full-term pregnancy
[f]Missing values were excluded from percentage calculations.

Table 4.2: Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with BI-RADS measured mammographic density by race

BI-RADS Categorized Density	Cases	Controls	Age and Race Adjusted OR (95% CI)[a]	Model 1 OR(95% CI)[b]	Model 2 OR(95% CI)[c]
All women					
Entirely fat	13	25	0.46 (0.22-0.96)	1.00 (Referent)	0.48 (0.22-1.08)
Scattered fibroglandular densities	183	197	1.00 (Referent)	2.07 (0.93-4.59)	1.00 (Referent)
Heterogeneously dense	232	253	0.97 (0.72-1.29)	2.06 (0.92-4.60)	1.00 (0.73-1.35)
Extremely dense	63	53	1.13 (0.71-1.78)	2.45 (0.99-6.09)	1.19 (0.72-1.95)
			P_{trend} = 0.24[d]	P_{trend} =0.24[e]	
White women					
Entirely fat	5	10	0.39 (0.12-1.26)	1.00 (Referent)	0.37 (0.10-1.35)
Scattered fibroglandular densities	98	108	1.00 (Referent)	2.68 (0.74-9.74)	1.00 (Referent)
Heterogeneously dense	144	171	0.90 (0.62-1.31)	2.50 (0.69-9.07)	0.93 (0.63-1.39)
Extremely dense	50	35	1.34 (0.77-2.36)	3.72 (0.94-14.81)	1.39 (0.75-2.55)
			P_{trend} = 0.24	P_{trend} =0.23	
African American women					
Entirely fat	8	15	0.49 (0.19-1.29)	1.00 (Referent)	0.49 (0.17-1.40)
Scattered fibroglandular densities	85	89	1.00 (Referent)	2.03 (0.71-5.77)	1.00 (Referent)
Heterogeneously dense	88	82	1.07 (0.68-1.67)	2.07 (0.71-6.03)	1.02 (0.63-1.66)
Extremely dense	13	18	0.76 (0.33-1.72)	1.53 (0.40-5.92)	0.75 (0.30-1.91)
			P_{trend} = 0.59	P_{trend} = 0.73	
Test of effect modification by race *P*=0.76					

[a]Adjusted for matching factors age and race. Models for all, African American, and white women were adjusted for age, and the model for all women was also adjusted for race.
[b]Model 1 for African American and white women is adjusted for age, BMI, menopausal status, family history of breast cancer, age at menarche, HT use, and parity and age at first full term pregnancy combined, where BI-RADS category 1 (almost entirely fat) is the referent group. Model 1 is additionally adjusted for race in all women.
[c]Model 2 is adjusted for the same variables as Model 1 but BI-RADS category 2 (scattered fibroglandular densities) is the referent group.
[d]*P* for trend test is based on likelihood ratio test statistic and is two-sided.
[e]*P* the same ordinal model was fit to assess the *p*-value of trend for Model 1 and Model 2.
BI-RADS, Breast Imaging Reporting and Data System

Table 4.3: Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with BI-RADS measured mammographic density by age, body mass index (BMI), and hormone therapy (HT) use

BI-RADS Categorized Density	Cases	Controls	Age and Race Adjusted OR (95% CI)[a]	Model 2 OR (95% CI)[b]
All women				
Entirely fat	13	25	0.46 (0.22-0.96)	0.48 (0.22-1.08)
Scattered fibroglandular densities	183	197	1.00 (Referent)	1.00 (Referent)
Heterogeneously dense	232	253	0.97 (0.72-1.29)	1.00 (0.73-1.35)
Extremely dense	63	53	1.13 (0.71-1.78)	1.19 (0.72-1.95)
			P_{trend} = 0.24[c]	P_{trend} = 0.24[c]
Current Hormone Therapy				
Yes				
Entirely fat	3	8	0.58 (0.14-2.46)	0.46 (0.08-2.53)
Scattered fibroglandular densities	39	69	1.00 (Referent)	1.00 (Referent)
Heterogeneously dense	70	97	1.25 (0.73-2.14)	1.13 (0.64-2.00)
Extremely dense	17	7	5.09 (1.83-14.16)	5.61 (1.86-16.96)
			P_{trend} =0.005	P_{trend} =0.01
No				
Entirely fat	10	17	0.41 (0.17-0.97)	0.49 (0.19-1.26)
Scattered fibroglandular densities	142	124	1.00 (Referent)	1.00 (Referent)
Heterogeneously dense	161	151	0.91 (0.64-1.30)	0.93 (0.64-1.34)
Extremely dense	46	44	0.72 (0.42-1.22)	0.80 (0.45-1.43)
			P_{trend} =0.82	P_{trend} =0.88

Test of effect modification by HT P=0.0002

Table 4.3 (continued): Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with BI-RADS measured mammographic density by age, body mass index (BMI), and hormone therapy (HT) use

BI-RADS Categorized Density	Cases	Controls	Age and Race Adjusted OR (95% CI)[a]	Model 2 OR (95% CI)[b]
Age				
<50				
Entirely fat	4	4	0.93 (0.20-4.20)	0.78 (0.16-3.77)
Scattered fibroglandular densities	56	66	1.00 (Referent)	1.00 (Referent)
Heterogeneously dense	110	114	1.14 (0.71-1.81)	1.10 (0.68-1.79)
Extremely dense	46	35	1.30 (0.71-2.39)	1.45 (0.75-2.79)
			=0.38	P_{trend} =0.27
50+				
Entirely fat	9	21	.5-0.87)	0.40 (0.15-1.04)
Scattered fibroglandular densities	127	131	eferent)	1.00 (Referent)
Heterogeneously dense	122	139	8-1.22)	0.92 (0.62-1.38)
Extremely dense	17	18	0.88 (0.41-1.89)	1.06 (0.46-2.40)
			P_{trend} =0.67	P_{trend} =0.46

Test of effect modification by age *P*=0.67

Table 4.3 (continued): Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer risk associated with BI-RADS measured mammographic density by age, body mass index (BMI), and hormone therapy (HT) use

BI-RADS Categorized Density	Cases	Controls	Age and Race Adjusted OR (95% CI)[a]	Model 2 OR (95% CI)[b]
Body Mass Index				
Women with BMI<25				
Entirely fat	1	5	0.11 (0.01-1.16)	0.12 (0.01-1.36)
Scattered fibroglandular densities	55	37	1.00 (Referent)	1.00 (Referent)
Heterogeneously dense	91	97	0.66 (0.38-1.14)	0.67 (0.38-1.19)
Extremely dense	36	30	0.70 (0.34-1.42)	0.75 (0.36-1.57)
			P_{trend} =0.64	P_{trend} =0.74
Women with BMI 25-29				
Entirely fat	5	5	0.99 (0.25-3.95)	1.36 (0.28-6.55)
Scattered fibroglandular densities	49	64	1.00 (Referent)	1.00 (Referent)
Heterogeneously dense	71	87	1.01 (0.60-1.70)	1.24 (0.70-2.20)
Extremely dense	16	17	1.14 (0.49-2.66)	1.35 (0.52-3.49)
			P_{trend} =0.82	P_{trend} =0.52
Women with BMI 30^{+}				
Entirely fat	7	15	0.45 (0.15-1.36)	0.43 (0.15-1.28)
Scattered fibroglandular densities	79	96	1.00 (Referent)	1.00 (Referent)
Heterogeneously dense	70	69	1.16 (0.72-1.87)	1.24 (0.76-2.04)
Extremely dense	11	6	2.52 (0.86-7.38)	3.29 (1.00-10.83)
			P_{trend} =0.03	P_{trend} =0.01
Test of effect modification by BMI *P*=0.09				

[a]Adjusted for matching factors, age and race.
[b]Model 2 is adjusted for age, race, BMI, menopausal status, family history age at menarche, HT, and parity and age at first full term pregnancy combined, where BI-RADS category 2 (scattere ensities) is the referent group.
[c]*P* for trend test is based on likelihood ratio test statistic and is two-sided.
BI-RADS, Breast Imaging Reporting and Data System

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CHAPTER 5: RESULTS

5.1 Introduction

Recent clinical studies on the molecular profiles of breast cancers have indicated that breast tumors can be classified into five etiologically and prognostically-relevant subtypes on the basis of gene expression patterns. Luminal A and Basal-like breast cancers have been emphasized in clinical and epidemiologic studies [1-5], with the former representing the largest percentage (45%) of cancers and having best prognosis, and the latter being rarer (5-15% of cases) with poorer survival outcomes [3, 6, 7]. Basal-like breast cancers are more prevalent among younger African American breast cancer cases and show unique risk factor patterns, often having risk factor specific associations that are in the opposite direction of what has been observed for breast cancer overall and Luminal A tumors [3]. For example, the protective effects of parity are observed with breast cancers overall and with luminal breast cancers, but appear to be reversed with Basal-like breast cancer; Basal-like breast cancer risk is higher among parous and multiparous women compared to nulliparous women [3]. Thus, stratification of risk factor analyses by subtype can identify etiologic heterogeneity of breast cancer.

Tumors of Basal-like and Luminal A subtypes have different immunohistochemical profiles and can be identified using paraffin embedded tissues in epidemiologic studies. Basal-like tumors are estrogen receptor (ER) negative, progesterone receptor (PR) negative, human epidermal growth factor receptor-2 (HER-2/*neu*) negative, and cytokeratin 5/6 and/or HER-1 positive; whereas Luminal A tumors are ER+, PR+, and HER-2/*neu*- [4]. In the Carolina Breast Cancer Study, breast cancer subtypes have been evaluated to

characterize the epidemiology of Basal-like breast cancer, but data on breast density were unavailable until recently. For the current investigation, participants in the Carolina Breast Cancer Study were matched to the Carolina Mammography Registry to allow estimation of the association between mammographic density and risk of specific breast cancer subtypes.

Mammographic breast density is a measure of the fibroglandular tissue composition in the breast and describes the radiological appearance of dense breast tissue. Different classification schemes have been used to visually characterize breast density, including Wolfe's parenchymal patterns [8, 9], Tabar's classification scheme [10], the American College of Radiology's Breast Imaging Reporting and Data System (BI-RADS) [11], as well as more quantitative methods that estimate the percentage of the breast that is dense. Reviews have concluded that mammographic density is associated with breast cancer risk regardless of the method used to measure breast density [12, 13]. In fact, breast density is one of the strongest and most consistent risk factors for breast cancer, and studies have estimated that women with the highest mammographic density may be at a 4-6 fold increased risk of developing breast cancer compared with women with the least dense tissue [12, 14-20]. However, there are conflicting results on the association between breast density and risk of breast cancer subtypes defined by hormone receptor status [21]. Of the six case-control and cohort studies examining the association between breast density and breast cancer risk by breast cancer hormonal status and/or subtypes to date as recently reviewed in Boyd *et. al.* [21], four observed increased risk of both ER+ and ER- tumors [22-25], and two observed increased risks for ER+ tumors only. Of the ten studies with cases only that examined whether breast density was different based on hormone receptor status, all but one [26] concluded that there were no significant differences in breast density by hormone receptor status [27-35].Although studies have examined the association between breast density and breast cancer risk by hormonal status, studies examining the association

between breast density and risk of specific intrinsic subtypes of breast cancer, particularly luminal and Basal-like subtypes, have been lacking [21].

We therefore examined the association between mammographic breast density and Basal-like and Luminal A subtypes of breast cancer.

5.2 Methods

5.2.1 Study setting and population

Subjects included in this study were participants in the CBCS who also had mammograms recorded in the CMR. CBCS is a population-based, case-control study designed to identify genetic and environmental factors for breast cancer risk in African American and Caucasian residents of 24 counties in North Carolina. The Carolina Mammography Registry (CMR) is a mammography registry that prospectively collects data from women and radiologists in mammography facilities in community practice, funded by the Department of Defense in 1994 and supported as part of Breast Cancer Surveillance Consortium by the National Cancer institute since 1995. Both CBCS and CMR are described in detail in Razzaghi *et al.* (Chapter 4).

Data from CBCS and CMR were combined to allow case-control and case-only analyses of breast density in association with breast cancer subtype. Briefly, CMR and CBCS were linked using probabilistic linkage with four variables; first and last name, date of birth, and last four digits of the social security number [36-38]. BI-RADS breast density, age and current use of hormone therapy at the time of the mammogram were collected from the CMR and all other participant data were taken from the CBCS. The following counties from the CBCS were not represented in our study because there were no matching cases and controls in the CMR: Alamance, Orange, Wake, Johnston, Lee, Harnett, Bertie, Wilson, Edgecombe, Pitt, Pamlico, Beaufort, and Tyrell.

5.2.2 Tumor blocks and immunohistochemistry assays

The details of breast cancer subtyping in CBCS have been published previously [3]. Briefly, all breast cancers underwent pathology review and descriptive data including type of biopsy, tumor size, laterality, and other characteristics were abstracted from pathology reports. Three H&E-stained slides were produced from each of the paraffin blocks when slices were made for molecular and immunohistochemical analyses (IHC). These slides were reviewed in a standardized fashion by the study pathologist to confirm the diagnosis of breast cancer and to assign histologic classification [39]. The following markers were used to determine breast cancer subtypes: Luminal A (ER+ and/or PR+, HER2-), Luminal B (ER+ and /or PR+, HER2+), Basal-like (ER-, PR-, HER2-, HER1+ and/or CK5/6+), HER2+/ER- (ER-, PR-, HER2+), and unclassified (negative for all five markers) [3, 4]. Only Luminal A and Basal-like are examined in detail in the current analysis due to the small number of HER2+ and Luminal B cases.

To determine ER/PR status, tumor blocks were sectioned and stained for a panel of IHC markers at the IHC Core Laboratory, University of North Carolina (UNC). Commercially available antibodies to ER, HER2, HER1, and Cytokeratin 5/6 were used in this study [4, 40, 41]. For invasive cases, ER/PR status was obtained from medical records for 80% of cases and determined using IHC assays performed at UNC for the remaining cases. For 11% of the cases with missing status for ER/PR on medical records, paraffin-embedded tissues were used and ER/PR status was determined at the UNC laboratory using IHC. For the remaining 9 percent of the cases, ER/PR status was missing [4, 39, 42].

5.2.3 Mammographic density assessment

Mammographic breast density is recorded qualitatively in the CMR using BI-RADS. BI-RADS density assessment defines four categories of breast composition including: 1) almost entirely fat, 2) scattered fibroglandular densities, 3) heterogeneously dense, and 4) extremely dense [43]. As discussed previously in Razzaghi *et al.*, density was the reported density from the screening or diagnostic mammogram performed within five years prior to or one year after breast cancer diagnosis for cases. Mammograms for controls were screening or diagnostic mammograms showing no cancer within five years prior to and three years after the selection date. The rationale for choosing a control group with a broader exposure window has been discussed previously (Chapter 4). For women with multiple mammograms, order of preference was (1) mammogram prior to breast cancer diagnosis or selection date into CBCS with date closest to diagnosis or selection date, (2) nearest mammogram after diagnosis/selection. Studies have shown that elevated risks of breast cancer associated with breast density persist for at least 5 years, with studies showing lasting effects for 10 years or more for both pre- and postmenopausal women [15, 19, 44-46]. Mammograms more than one year following treatment were excluded based on suggestions in the literature that agents used to treat breast cancer may alter breast density as early as 18 months after initiating therapy [47]. Breast density measured in the CMR is per woman and not per breast. Vachon *et al.* concluded that density is a general marker of breast cancer risk and is not specific to breast side or location of the eventual cancer [48]; density has also been shown to be highly correlated between breasts within a woman [49].

5.2.4 Statistical Analysis

The variable coding schemes were chosen for consistency with previous CBCS publications [3]. Briefly, race was categorized as African American or white based on self-report. Mammographic breast density was based on the four BI-RADS density categories. Age at diagnosis was used for cases and age at selection into the CBCS for controls and was analyzed as a continuous variable. Body mass index (BMI) was calculated as body weight (kg)/height $(m)^2$ and was treated as a continuous variable in the analysis. Age at first full-term pregnancy and parity/nulliparity were combined to create a categorical variable that encapsulated both parity status and age at first birth. Because of the association between age, hormone therapy use, and breast density, we also examined age and current hormone therapy at the time of the mammogram recorded in the CMR as explained in detail in our previous study (Chapter 4). Hormone therapy (HT) was categorized as current or not-current as collected by the CMR at the time of the mammogram. All categorical variables were coded using indicator variables.

We used unconditional logistic regression with breast cancer as the outcome variable to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between breast density and breast cancer risk (SAS version 9.3, SAS Institute, Cary NC). We also examined risk of triple-negative breast tumors [estrogen (ER), progesterone (PR), and human epidermal growth factor receptor-2 (HER-2) negative tumors] according to breast cancer subtype to facilitate direct comparisons with the only other study on the association between breast density and risk of breast cancer subtypes. Case-case analyses were used to compare odds of breast density across subtypes, comparing Basal-like to Luminal A and triple-negative to Luminal A breast cancers.

Effect measure modification was not assessed in this study given the small sample size. Potential confounders were selected based on prior knowledge and using directed acyclic graphs (DAGs) [50]. We adjusted for age, race, BMI, HT, menopausal status, first degree family history of breast cancer, age at menarche, and parity and age at first full-term pregnancy (with the latter two combined into a single variable). We also adjusted for the offset term used in the CBCS to account for oversampling of young African American women [51].

5.3 Results

Characteristics of all cases (491 breast cancer cases) and women with Basal-like and Luminal A tumors as well as 528 controls are presented in Table 1. Women with Basal-like subtype were younger, had higher BMI and WHR, were more likely to be African American, premenopausal, younger than 13 at menarche, parous with first full term pregnancy at younger than 26, not current HT users, users of oral contraceptives, and never breastfeeders compared to women with Luminal A breast cancer (Table 1). Thus, associations with standard risk factors showed similar patterns by subtype as reported for the CBCS overall [3].

Table 2 presents the ORs and 95% CIs for adjusted models with both BI-RADS 1 (Model 1) and 2 (Model 2) as the reference groups. Model 1 is included to facilitate comparison with previous studies that have reported risk for the 'extremely dense', BI-RADS 4 group relative to 'entirely fatty', BI-RADS 1 group, but Model 2 allows for more precise estimates due to a larger referent group. Among all women, those with extremely dense breasts had an increased risk of breast cancer compared to women with scattered fibroglandular densities and those with entirely fatty breasts [1.19 (0.72, 1.95), and 2.45 (0.99, 6.09), respectively] (Table 2). Both estimates are imprecise. Model 1 resulted in a

stronger positive association between breast density and breast cancer risk for the Basal-like subtype compared to the Luminal A subtype [3.58 (0.34-37.97), and 1.98 (0.54-7.34), respectively]. These associations were of weaker magnitude when using Model 2, and importantly, associations were of similar magnitude for the Basal-like and Luminal A subtypes [1.04 (0.34-3.17), and 0.98 (0.50-1.92), respectively] (Table 2).

To facilitate comparisons with the only other study of breast density by breast cancer subtype [22], we also examined the association between breast density and breast cancer risk in case-control analyses using the "triple-negative" definition of breast cancer Model 1 resulted in a large, imprecise estimate for risk of triple negative breast cancer, and Model 2 resulted in a higher odds ratio than previously observed for either Basal-like or Luminal A breast cancers [1.20 (0.49-2.90)] (Table 2). We further compared the strength of associations between Basal-like and Luminal A breast cancers as well as the triple-negative and Luminal A breast cancers, using case-only analyses for model 2 (Table 3). In relation to breast density, there was no statistically significant difference between Basal-like and Luminal A or between triple-negative and Luminal A breast cancers [1.08 (0.30-3.84), and 1.17 (0.41-3.35), respectively], although the estimates are imprecise. Thus, based on our findings, there was no suggestion of etiologic heterogeneity with respect to breast density and subtype.

5.4 Discussion

Of the six case-control and cohort studies examining the association between breast density and breast cancer risk as reviewed in Boyd et. al. [21], four of six observed increased risk for both ER+ and ER- tumors [22-25, 52, 53], with only two showing that ER- tumors were less commonly associated with breast density [52, 53]. Both quantitative and qualitative measures of breast density were used in these studies, but these studies

evaluated only hormone receptor status (primarily ER only, with only Conroy *et al.* [53], Yaghjyan *et al.* [25], and Ma *et al.* [22] including both estrogen and progesterone receptor status). A plausible explanation for the conflicting results regarding ER negative tumors is that ER negative tumors are heterogeneous, including HER2 positive, basal-like, and unclassified tumors. Therefore, we evaluated the breast density-breast cancer association using more definitive subtype markers. Using these five markers, we observed no difference in the breast density-breast cancer association for Luminal A, Basal-like, or triple-negative breast cancers based on case-control analyses. Furthermore, our estimates from case-only analysis, which can be interpreted as ratios of ORs between the two subtypes of breast cancer (Luminal A and Basal-like subtypes), directly estimated the relative strength of association between the two breast cancer subtypes and findings were not statistically different from the null, concluding no significant difference between Basal-like and Luminal A or triple-negative and Luminal A breast cancers.

To our knowledge, no previous study has examined the association between breast density and risk of breast cancer subtypes using five markers to classify breast cancer subtypes (ER, PR, HER2, EGFR, and ck5/6). Use of five IHC markers reduces concerns about heterogeneity among triple negative breast cancers; the class of triple-negative breast cancers includes some tumors where all assays failed and therefore has higher levels of outcome misclassification (with some tumors of other subtypes inappropriately grouped with Basal-like subtype) [54]. Our results from the case-only analysis were similar to the other case-only studies of this association. Nine of the ten case-only studies that examined whether breast density was different based on hormone receptor status concluded that there were no significant differences in breast density by hormone receptor status [27-35]; only two of these studies (both null) examined the association using breast cancer subtypes [30, 31]. Outcome misclassification of this sort

would lead to bias toward the null and could explain previous as well as our findings of no case-only odds ratio modification by subtype. Our findings can be interpreted, together with our case control analyses showing that breast density is weakly positively associated with both Luminal A and Basal-like breast cancers, as evidence that breast density is a risk factor for both subtypes with no evidence of heterogeneity by tumor subtype. Thus, outcome misclassification and resulting bias toward the null (and type two error) seems to be an unlikely explanation for the previous findings of no effect modification by ER or triple-negative subtype.

In addition to our study, one previous study has included use of HER2 status in classifying tumors, comparing ER+ to triple-negative breast cancers [22]. This study included 184 cases of Luminal A (defined ER+, PR-, and HER2-) and 106 cases of triple-negative breast cancers and found, in concordance with our results, that percent mammographic density was positively associated with both Luminal A [2.22 (1.04-4.78)] and triple-negative [2.96 (1.21-7.23)] breast cancer [22]. Given the concordance of our findings with those of Ma *et al*. it is possible that there are genetic and heritable factors that alter breast density and breast cancer risk, and are therefore responsible for the association of breast density and breast cancer regardless of breast cancer subtypes [55]. For example, heritable differences in exposure or response to hormones and growth factors may increase proliferative activity and quantities of stromal and epithelial tissue, with effects on both breast density and breast cancer risk [55, 56]. Consistent with this, a recent study has demonstrated that two of 14 established breast cancer loci simultaneously contribute to large between-woman differences in mammographic density [57]. This model, wherein breast density serves as a marker of hormonal and other influences on breast tissue composition, is also supported by work examining breast density and non-genetic breast cancer risk factors. Hormonal

exposures, such as parity and HT, for example, have strong associations with breast density and are similarly associated with breast cancer risk [58]. Additionally, we, along with others, have concluded that the association between breast density and breast cancer risk is stronger among overweight and obese women as well as women with current use of HT (Chapter 4); given the strong correlations between these two risk factors and breast density, BMI and HT could be responsible for this strong association through their effects on breast density.

Some of these well known breast cancer risk factors have opposite effects on Basal-like and Luminal A subtypes of breast cancer [3]. For example, Millikan *et al.* identified risk factors for the Basal-like subtype including younger age at diagnosis, higher parity, younger age at first full-term pregnancy, shorter duration of breastfeeding, fewer number of children breastfed, fewer number of months breastfeeding per child, and increased waist-to-hip ratio [3]. Because many of these variables that have distinct associations with breast cancer subtypes also impact breast density, we might have expected to see differences in the breast density-breast cancer subtype association. For example, young age at first full-term pregnancy is associated with lower breast density [59] and a reduction in risk for Luminal A breast cancers [44]. However, it appears that breast density does not have an association with subtypes that is independent of these factors. In our models that controlled for these as covariates or confounders, there was no evidence of heterogeneity of the breast density-breast cancer association by subtype.

In summary, major strengths of our study were inclusion of the five markers to identify breast cancer subtypes (ER, PR, HER2, HER1 and CK5/6). However, as with other studies of breast density by molecular subtype, the main limitation of our study was small sample size leading to wider confidence intervals. As a result of smaller sample size, we were underpowered to study effect measure modification by race and hormone

therapy. Future studies with breast cancer subtypes and breast density by race are desirable, particularly given that Basal-like breast cancers are more prevalent in African American women and appear to have distinct etiology. However, based on current data, there is little evidence to support differences in the effect of breast density by breast cancer subtype.

5.5 Tables

Table 5.1: Population characteristics by tumor subtype, Basal-like and Luminal A breast cancers

Variable	Overall cases vs. controls		Basal-like		Luminal A	
	Cases	Controls	N	OR (95% CI)	N	OR (95% CI)
No of subjects	491	528	48		181	
Mean age (CBCS), y[a]	53.2 (28-74)	54.0 (31-74)	50.2 (33-73)	0.99 (0.96-1.02)	54.5 (31-74)	1.04 (1.02-1.06)
Mean BMI	28.6 (15.1, 60.6)	28.8 (14.6, 60.9)	30.9 (19.1-44.2)	1.06 (1.02-1.10)	28.5 (15.0-52.6)	1.00 (0.98-1.03)
Mean number of days[b]	-21 (-1401, 365)	149 (-1617, 1095)	-27 (-938, 365)		-10 (-1050, 365)	
Race						
White	297 (60.5%)	324 (61.4%)	21 (43.8%)	1.00	116 (64.1%)	1.00
African American	194 (39.5%)	204 (38.6%)	27 (56.3%)	3.32 (1.80-6.12)	65 (35.9%)	1.31 (0.90-1.89)
Menopausal status						
Premenopausal	200 (40.7%)	213 (40.3%)	25 (52.1%)	1.00	67 (37.0%)	1.00
Postmenopausal	291 (59.3%)	315 (59.7%)	23 (47.9%)	0.90 (0.49-1.65)	114 (63.0%)	1.83 (1.27-2.65)
Family history[c]						
No	386 (81.1%)	440 (85.6%)	39 (83.0%)	1.00	149 (84.2%)	1.00
Yes	90 (18.9%)	74 (14.4%)	8 (17.0%)	1.24 (0.55-2.82)	28 (15.8%)	1.11 (0.67-1.84)
Missing	15	14	1		4	
Age at menarche						
<13	257 (52.3%)	230 (43.6%)	32 (66.7%)	1.00	92 (50.8%)	1.00
≥13	234 (47.7%)	298 (56.4%)	16 (33.3%)	0.37 (0.19-0.70)	89 (49.2%)	0.76 (0.53-1.09)
Parity & Age at FFTP						
Nulliparous	74 (15.1%)	67 (12.7%)	6 (12.5%)	1.00	31 (17.1%)	1.00
Parous, <26	312 (63.5%)	347 (65.7%)	36 (75.0%)	2.07 (1.04-4.15)	107 (59.1%)	0.93 (0.64-1.34)
Parous, 26+	105 (21.4%)	114 (21.6%)	6 (12.5%)	0.43 (0.18-1.06)	43 (23.8%)	0.96 (0.63-1.47)
Breastfeeding						
Never	299 (60.9%)	324 (61.4%)	32 (66.7%)	1.00	110 (60.8%)	1.00
Ever	192 (39.1%)	204 (38.6%)	16 (33.3%)	0.84 (0.44-1.60)	71 (39.2%)	1.09 (0.75-1.57)
Lifetime duration lactation						
Never	299 (60.9%)	324 (61.4%)	32 (66.7%)	1.00	110 (60.8%)	1.00
>0-3 months	72 (14.7%)	69 (13.1%)	9 (18.8%)	1.71 (0.77-3.79)	26 (14.4%)	1.14 (0.68-1.92)
4+ months	120 (24.4%)	135 (25.6%)	7 (14.6%)	0.50 (0.22-1.16)	45 (24.9%)	1.02 (0.67-1.55)
Current HT use[d]						
Yes	129 (26.4%)	181 (35.0%)	9 (18.8%)	1.00	43 (23.9%)	1.00

No	359 (73.6%)	336 (65.0%)	39 (81.2%)	2.36 (1.11-5.05)	137 (76.1%)	1.84 (1.23-2.77)
Missing	3	11	0		1	
Oral contraceptive use						
Never	170 (34.6%)	170 (32.4%)	11 (22.9%)	1.00	72 (39.8%)	1.00
Ever	321 (65.4%)	355 (67.6%)	37 (77.1%)	1.21 (0.59-2.46)	109 (60.2%)	0.49 (0.34-0.71)
Missing	0	3	0		0	
WHR						
<0.77	132 (27.3%)	169 (32.3%)	4 (8.7%)	1.00	45 (25.4%)	1.00
0.77-0.83	171 (35.3%)	173 (33.0%)	17 (37.0%)	1.19 (0.63-2.24)	69 (39.0%)	1.41 (0.97-2.05)
≥0.84	181 (37.4%)	182 (34.7%)	25 (54.3%)	2.40 (1.30-4.42)	63 (35.6%)	1.17 (0.80-1.71)
Missing	7	4	2		4	

[a]Mean age at diagnosis for cases and selection for controls in the CBCS
[b]Mean number of days between diagnosis date for cases and selection date for controls in the CBCS and the date of the mammogram chosen to assess breast density
[c]First-degree family history of breast cancer
[d]Current hormone therapy (HT) use at the time of the mammogram
OR, odds ratio. CI, confidence interval. CBCS, Carolina Breast Cancer Study. BMI, body mass index. FFTP, first full-term pregnancy. HT, hormone therapy. WHR, waist-to-hip ratio.

Table 5.2: Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for breast cancer risk by tumor subtype associated with BI-RADS measured mammographic density

	Cases vs. Controls					Triple Negatives vs. Controls	
	Controls	Cases	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)	TN cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Almost Entirely Fat	25	13	1.00 (Referent)	0.48 (0.22-1.08)	1	1.00 (Referent)	0.17 (0.02-1.43)
Scattered Fibroglandular Densities	197	183	2.07 (0.93-4.59)	1.00 (Referent)	31	5.96 (0.70-50.64)	1.00 (Referent)
Heterogeneously Dense	253	232	2.06 (0.92-4.60)	1.00 (0.73-1.35)	40	5.83 (0.68-50.04)	0.98 (0.55-1.75)
Extremely Dense	53	63	2.45 (0.99-6.09)	1.19 (0.72-1.95)	12	7.13 (0.74-68.90)	1.20 (0.49-2.90)
			P_{trend} =0.24[c]			P_{trend} =0.31	

Table 5.2: Continued: Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for breast cancer risk by tumor subtype associated with BI-RADS measured mammographic density

	Basal-likes vs. Controls			Luminal As vs. Controls		
	BL cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)	LA cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Almost Entirely Fat	1	1.00 (Referent)	0.29 (0.03-2.51)	4	1.00 (Referent)	0.49 (0.15-1.59)
Scattered Fibroglandular Densities	19	3.45 (0.40-29.90)	1.00 (Referent)	69	2.03 (0.63-6.59)	1.00 (Referent)
Heterogeneously Dense	22	3.03 (0.34-26.67)	0.88 (0.43-1.80)	86	2.09 (0.64-6.79)	1.03 (0.68-1.56)
Extremely Dense	6	3.58 (0.34-37.97)	1.04 (0.34-3.17)	22	1.98 (0.54-7.34)	0.98 (0.50-1.92)
		P_{trend} =0.67			P_{trend} =0.60	

[a]Model 1 is adjusted for age, race, BMI, menopausal status, family history of breast cancer, age at menarche, HT, and parity and age at first full term pregnancy combined, where BI-RADS category 1 (almost entirely fat) is the referent group.
[b]Model 2 is adjusted for the same variables as Model 1 but BI-RADS category 2 (scattered fibroglandular densities) is the referent group.
[c]*P* for trend test is based on likelihood ratio test statistic and is two-sided. The same ordinal model was fit to assess the *p*-value of trend for Model 1 and Model 2.
Bi-RADS, Breast Imaging Reporting and Data System. TN, triple negative. BL, Basal-like. LA, Luminal-A.

Table 5.3: Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for case-case analyses comparing the association with BI-RADS measured mammographic density by breast cancer risk subtypes

	Basal-likes vs. Luminal As		Triple-negatives vs. Luminal As	
	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Almost Entirely Fat	1.00 (Referent)	1.05 (0.10-10.97)	1.00 (Referent)	0.33 (0.03-3.95)
Scattered Fibroglandular Densities	0.95 (0.09-9.90)	1.00 (Referent)	3.05 (0.25-36.68)	1.00 (Referent)
Heterogeneously Dense	0.63 (0.06-6.65)	0.67 (0.30-1.49)	2.62 (0.22-31.62)	0.86 (0.44-1.67)
Extremely Dense	1.02 (0.08-13.50)	1.08 (0.30-3.84)	3.57 (0.26-49.11)	1.17 (0.41-3.35)
	P_{trend} =0.66[c]		P_{trend} =0.74	

[a]Model 1 is adjusted for age, race, BMI, menopausal status, family history of breast cancer, age at menarche, HT, and parity and age at first full term pregnancy combined, where BI-RADS category 1 (almost entirely fat) is the referent group.
[b]Model 2 is adjusted for the same variables as Model 1 but BI-RADS category 2 (scattered fibroglandular densities) is the referent group.
[c]*P* for trend test is based on likelihood ratio test statistic and is two-sided. The same ordinal model was fit to assess the *p*-value of trend for Model 1 and Model 2.
BI-RADS, Breast Imaging Reporting and Data System

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CHAPTER 6: SUMMARY AND CONCLUSIONS

6.1 Main findings

The purpose of this dissertation was to examine the associations between breast density, race, and risk of intrinsic subtypes of breast cancer risk. Modification of the odds ratio for the breast density-breast cancer was evaluated by age, race, BMI, and current hormone therapy use in breast cancers overall, and odds ratios for the breast density-breast cancer association were estimated among Basal-like and Luminal A breast cancers (defined as ER-, PR-, HER2-, HER1-, and/or CK 5/6+ for Basal-like and ER+, PR+, and HER2- for Luminal A breast cancers).

Increasing mammographic breast density was associated with increased breast cancer risk in CBCS, where women with extremely dense breasts had a nearly 3-fold increased risk of breast cancer compared with women with breasts composed of almost entirely fat tissue. The odds ratio estimates were similar to those previously reported using BI-RADS measurement of mammographic breast density [1]. Due to small sample size of BI-RADS density category 1, BI-RADS density category 2 was also used as the reference group to estimate risk which resulted in more precise effect estimates, but with lower magnitude than reported previously [2]. Although race did not significantly modify the association between breast density and breast cancer risk, we found effect estimates that were substantially weaker for African American women compared with white women; additionally we observed differences in the distributions of breast density between white and African American women with breast density being lower in African American women which

was consistent with two other studies [3, 4]. We further examined BMI and HT as effect measure modifiers and found that odds ratios were greater for obese women compared with non-obese women and among current HT users compared with non-current HT users.

To assess the potential for etiologic heterogeneity among breast cancer subtypes, the associations between breast density and Basal-like and Luminal A breast cancers were estimated. Basal-like subtype was defined using five markers, based on concerns that triple-negative breast cancers may not specifically capture Basal-like subtype [5]. We found that breast density was weakly positively associated with both Luminal A and Basal-like breast cancers, with no evidence of heterogeneity of the breast density-breast cancer association by tumor subtype. When using BI-RADS category 1, stronger associations were observed for Basal-like tumors; however, these estimates were imprecise given the small sample size. These results were consistent with the only previous study to have evaluated the association to date, which showed little or no evidence of etiologic heterogeneity across subgroups of breast cancers [6]. Together these results demonstrate a positive association between breast density and breast cancer risk regardless of breast cancer subtype.

6.2 Strengths and limitations

6.2.1 Strengths

This study combined two rich data sets -- the CBCS, where young African American women were oversampled, and the CMR -- to examine the associations between breast density, race, and breast cancer risk with respect to modification of the breast density-breast cancer risk relationship by age, race, BMI, and current hormone therapy as well as the association between breast density and Basal-like and Luminal A breast cancers. A major strength of our study is that we used 5 markers to identify breast

cancer subtypes (ER, PR, HER2, HER1 and CK5/6); using these 5 markers we were able to identify Basal-like breast cancers rather than grouping these cancers into a broader definition of triple-negative breast cancers (ER, PR, and HER2 negative). Oversampling of young African American women, who also have the highest prevalence of Basal-like breast cancers, also increased our power to evaluate the effects of breast density on risk of specific subtypes. The results of these analyses have provided new data to elucidate the role of breast density in breast cancer disparities.

6.2.2 Limitations

While the use of data from the CMR linked BI-RADS breast density data with breast cancer and other demographic factors, some limitations resulted due to collection of data at different time points. For some women mammograms were not available within one year prior to their diagnosis or selection date into the CBCS. To maximize our sample size, we used mammograms within five years prior to and one to three years post-diagnosis and selection dates, with support from studies showing that this wider window is as predictive of breast cancer risk as using mammograms one year prior to breast cancer diagnosis. By also using some of the variables such as hormone therapy from the CMR, we were able synchronize important covariates with the main exposure. Additionally, our study used a qualitative measure of breast density rather than a quantitative measure (percent mammographic density), which could have attenuated our results. However, this measurement method should not have had substantial influence on the results observed since previous studies have shown that BI-RADS breast density is predictive of breast cancer risk but not as strong predictor as the quantitative measures [7] and since our findings were similar to the only other study on the association of interest [6].

Few studies are adequately powered to evaluate mammographic density in African Americans, and while ours was a large study, some of our estimates were imprecise due to small numbers of women in the categories with strongest effects (e.g. extremely dense breasts). This was further compounded when stratifying on breast cancer subtype. As with many other studies of risk factor associations by molecular subtype, the main limitation of our study was small sample size leading to wider confidence intervals and inability to study effect measure modification by race and hormone therapy. Future studies with larger numbers of women as well as a full marker panel including the specific markers for Basal-like breast cancer should be conducted. Given the small sample size, we were unable to examine effect measure modification by age, BMI, and hormone use within the strata of race, nor could we assess the association between breast density and Basal-like and Luminal A breast cancers within the strata of race.

6.3 Public health impact

There are well-established racial differences in breast cancer incidence and survival [8]. Basal-like tumors are more prevalent among premenopausal African American women, and are associated with poorer survival compared to hormone receptor positive subtypes [9, 10]. Breast density is a strong risk factor for breast cancer, but the mechanism of its association with breast cancer as well as with breast cancer subtypes risk is poorly understood. While there is not a single mechanism or explanation that is implicated in the association between breast density and breast cancer risk [3, 4, 11-18], a stronger association between breast density and Basal-like breast cancers may be expected because many of the environmental and genetic factors that affect the risk of Basal-like breast cancer (young age, race, breastfeeding, parity) also affect breast density. Any genetic or environmental exposure that alters the proliferative activity and

quantity of epithelial and stromal tissue of the breast may influence density and/or breast cancer risk [19, 20]. The identification of risk factors for Basal-like breast cancer will help in identifying prevention strategies for this aggressive disease.

6.4 Future directions

This study suggests that the association between breast density and breast cancer risk may be modified by race, but the ability to precisely quantify this has been limited by sample size. Larger studies and meta-analyses will be needed to definitively answer this question. Additionally, by simultaneously considering effect modification by both race and race-associated variables, our study suggested important relationships between breast cancer risk factors and breast density. The role of BMI in modifying risk associated with breast density merits further investigation and is an important consideration in studies of breast density by racial/ethnic group. While the precise mechanism underlying these associations remains to be determined, the interaction between BMI and breast density may reflect underlying changes in breast tissue composition. Additionally, larger studies should examine the association of interest within racial groups or groups defined by hormone therapy use.

This study was also one of two case-control studies to have examined the association between breast density and risk of breast cancer subtypes and the only one that used 5 molecular markers to identify Basal-like breast cancers. Although we did not find substantial differences in the association between breast density and Basal-like and Luminal A breast cancers, future studies with larger numbers of Basal-like breast cancers are needed to further examine this association using the lowest breast density categories as the referent group as well as assessing effect measure modification by race, hormone therapy, and other important breast cancer risk factors.

This work focused on race and breast cancer subtypes, with the goal of identifying whether breast density contributes to disparities in breast cancer incidence. Lower breast density in African Americans, together with little evidence for modification by race in our study population, suggests that breast density does not lead to greater risk of breast cancer among African Americans. Furthermore, results from the second aim, estimating the effect of density in risk of Basal-like and Luminal A breast cancers, was also informative in that there was little evidence to support differences in the effect of breast density by breast cancer subtype. Together these results suggest that while breast density is an important risk factor for breast cancer overall, it may not play a major role in public health strategies focused on reducing breast cancer disparities. However, our work emphasizes that breast density is a strong risk factor for breast cancer and it is crucial to understand the mechanism of how breast density affects breast cancer risk.

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